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Epidemiology of antimicrobial resistance at the livestock-human interface in an urban environment: a One Health approach

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To my fabulous parents Isaac and Regina and late grandparents Grace and Dishon.

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Declaration

I declare that this thesis and the analyses described within it are original and my own composition, expect where explicitly stated below, and that this work has not been submitted for any other degree or professional qualification. Data collection described herein was primarily carried out by myself and members of the UrbanZoo project. Laboratory based work, including bacterial culture and bacterial DNA extraction were conducted by laboratory teams at University of Nairobi, Kenya Medical Research Institute, and the International Livestock Research Institute. Whole Genome Sequencing (WGS) of *E. coli* isolates was performed at the Wellcome Centre for Human Genetics, Oxford Genomics Centre (funded by Wellcome Trust grant reference 203141/Z/16/Z). Bioinformatic processing of sequences was conducted by members of the Modernizing Medical Microbiology Informatics Group (University of Oxford), Dr Melissa Ward, Dr Bryan Wee and myself. Phylogenetic analysis was conducted by Dr Bryan Wee and myself.

Dishon M Muloi

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Abstract

Livestock have been implicated as a reservoir for antimicrobial resistant (AMR) bacteria that may spread to humans, with the keeping of livestock widely postulated as a risk factor for AMR in humans. However, quantitative evidence of the role of livestock in the emergence and transmission of AMR bacteria to human populations is lacking. This thesis focuses on the role of livestock keeping as a potentially high-risk interface for AMR transmission between humans and livestock in urban Nairobi. To achieve this, *E. coli* isolates were systematically collected from sympatric human and livestock populations in 99 households across Nairobi, Kenya. *E. coli* was characterised both phenotypically (through antimicrobial susceptibility testing) and genetically (through whole genome sequencing).

In the first part of this thesis, I conduct a comprehensive systematic review to investigate existing evidence that food animals are responsible for transfer of resistant *E. coli* and their AMR determinants to humans. I demonstrate that the current evidence regarding transmission of drug resistance between food animals and humans is limited and that similarity of AMR bacteria or AMR determinants in the two populations does not, by itself, provide information on directionality of transfer. I highlight the need to use high resolution genomic analysis on human and livestock bacterial samples collected in time and space to better understand the direction and frequency of AMR transmission between these populations.

Next, utilising AMR phenotypes and genotypes, I explored the variation in carriage of AMR *E. coli* and investigated the role of livestock ownership as a risk factor for AMR carriage in humans. First, I explored the epidemiology of clinically relevant AMR phenotypes and AMR genetic markers. I detected high rates of AMR phenotypes, with 47.6% and 21.1% of isolates displaying resistance to ≥ 3 and ≥5 antimicrobial classes respectively. Whole-genome sequencing revealed 60 acquired genes and 14 point mutations conferring AMR to 9

antimicrobial classes. sul2, strA, strB, tetA, and blaTEM-1B were the most frequently detected AMR genes conferring resistance to sulfonamides, aminoglycosides, tetracyclines, and β-lactams respectively – the most commonly found phenotypes. Highest carriage of AMR genes and phenotypes was observed in humans, pigs and poultry compared to goats, rabbits and bovines. Secondly, I demonstrated that the presence of livestock in the household did not influence phenotypic or genotypic AMR carriage in humans, but the impact of keeping livestock on human AMR carriage was instead influenced by presence of animal manure in the household.

Utilising high resolution sequencing data, I proceeded to investigate the patterns of bacterial relatedness and strain sharing as a proxy for transmission potential. I showed that livestock and human isolates are genetically heterogeneous, with minimal evidence of clustering by host group, and that *E. coli* genomes in humans did not segregate according to livestock ownership. Next, I found evidence of 91 sharing events differing by less than ten base pairs (59 involving livestock isolates only 23 human isolates only, and 9 between humans and livestock), and that most of the sharing events were confined within households with only occasional instances of spread between household. I also demonstrate that high-resolution sequence-based analysis of SNPs is more discriminatory than MLST – a widely used tool in describing transmission of *E. coli*.

Next, I described the patterns of antimicrobial sales in humans and livestock, and the level of awareness and common behaviours related to antimicrobial prescribing amongst human and veterinary pharmacists in urban Nairobi. β-lactams, fluoroquinolones, first and second generation cephalosporins, and metronidazole were the most commonly purchased human antimicrobials while tetracyclines, sulphonamides, penicillins, and macrolides were the most commonly purchased veterinary antimicrobials. This finding was in line with the resistance phenotypes and genotypes described in this thesis. I found that

whilst most pharmacists were knowledgeable about antimicrobial use and AMR, inappropriate prescribing practices were common and that over the counter sale of antimicrobials, without a prescription, was a common occurrence in both human and veterinary drug stores.

In the final section of the thesis, I investigated the co-occurrence patterns of acquired AMR genes and the role of conjugative plasmids on the epidemiology of AMR spread. I found evidence of co-location of multiple AMR genes in both human and livestock isolates, potentially enabling acquisition and dissemination of multi-drug resistance phenotypes in a single step. I found a diversity of known plasmids and plasmid replicons that were associated with the distribution of acquired AMR genes.

To conclude, I discuss the findings of this thesis in the context of the current epidemiology of AMR pathogens at the human-livestock interface and highlight future directions for research on AMR transmission, and discuss implications of my findings for public health. This thesis demonstrates how fine-scale genomic analysis explicitly embedded within an epidemiologically structured sampling framework can be utilized to track bacterial sharing and in the surveillance of AMR prevalence in a low income urban setting. The connectivity of bacteria and their AMR determinants between humans and livestock and the ultimate impacts upon human health lends strong support for a holistic 'One Health' perspective for AMR surveillance.

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Lay Summary

Antimicrobial resistance (AMR) – the ability of bacteria to survive in the presence of antimicrobials – is a serious public health concern in all countries across the globe. The role of livestock as a source of antimicrobial resistant bacterial infections increasingly observed in humans has been the subject of much speculation and is poorly understood. This thesis investigates the transmission of AMR bacteria between human and livestock populations in a developing country urban environment using a One Health approach – defined as "an interdisciplinary approach that considers the fundamental connections between human, animal, and environmental health".

A common bacterium that inhabits the mammalian gut, *Escherichia coli (*hereafter *E. coli),* was isolated from faecal samples of cohabiting human and livestock populations across Nairobi, Kenya. *E. coli* were cultured and isolates were tested for resistance to a range of antimicrobials. The genomic information for bacterial isolates from each individual (complete set of DNA, including all of their genes) was obtained using a genetic analysis technique known as whole genome sequencing.

In the first part of this thesis, I carried out a systematic review of current evidence for the transfer of resistant *E. coli* between farm animals and people across the globe. I found studies suggesting that resistant *E. coli* bacteria can be passed directly to humans by livestock, but limitations in those studies mean that the causative role of farm animals in the rise and spread of AMR bacteria cannot be unequivocally determined.

Next, I analysed the occurrence of clinically relevant resistant bacteria in livestock and humans in Nairobi and the genes associated with this resistance. I observed a high prevalence of bacterial strains resistant to commonly used antimicrobials: sulfonamides, aminoglycosides, tetracyclines, and β-lactams. Resistant bacteria were most commonly found in humans, pigs and poultry, compared to other species such as cattle, goats and rabbits. These livestock

species are growing in importance in most developing countries. In addition, resistant bacteria were as common in people living in households without livestock as those in households with livestock. Also, disposing of animal manure in household compounds made it more likely that people would carry AMR bacteria. Further, my analysis suggested that resistance genes were mostly located on genetic elements (known as plasmids) that can be transferred both from one bacterium to another and between humans and animals, suggesting a mechanism for the spread of the causes of resistance.

Next, examining the bacterial genetic sequences from humans and livestock, and comparing them with each other, I found evidence of sharing of bacteria, mostly confined within humans, and separately, within livestock, with limited evidence of sharing between these groups of potential hosts. I also demonstrated that whole genome sequencing is more powerful in tracking sharing of bacteria in host populations than standard genetic tests that inspect only a handful of genes.

Finally, investigating the practices and knowledge among antimicrobial retailers in Nairobi, I found that, while most pharmacists were knowledgeable about antimicrobial use, inappropriate prescribing practices were common and that both human and veterinary antimicrobials were sold over the counter, often without a prescription.

This thesis demonstrates how robust data and state-of-the-art genome analysis can be used to shed light on how antimicrobial resistant bacteria arise and spread in human and animal populations, and my results can help design plans for preventing the rise of AMR as a public health problem.

Acknowledgements

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As it becomes a distant memory, suffices to say, innumerable people have made an indelible mark on my life over the fours that I have been working on this PhD. Firstly, I would like to say how very grateful I am to my supervisors Professor Mark Woolhouse, Dr Amy Pedersen, Professor Eric Fèvre, and Dr Melissa Ward. I feel particularly indebted to Mark, who provided me with the opportunity to undertake a PhD in Scotland, and for the support, advice, insightful ideas and feedback throughout this PhD. I couldn't have done this without you, Mark – thank you. I am hugely thankful to Amy for the productive conversations which were integral in shaping my ecology knowledge. Thank you to Melissa for sharing your microbial genetics expertise and the thought provoking discussions and guidance. Eric, thanks for not only being an academic mentor, but as a counsellor and a friend – thanks for believing in me, supporting me on this part of my academic journey, to my eternal gratitude.

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I am grateful to my friends, both in Edinburgh, and abroad for their support throughout the PhD. And, to single some individuals out – Hussein, Lian, Samir, Ammad, Joy, Welcome, Analyn, Charity, Katie, Bridgit, Patrick, Lisa, Adam, and Moses – thanks for the support or just generally been awesome.

My heartfelt thanks to my parents, Isaac Mwongela, Regina Muloi, my siblings, Joshua and Grace, and my larger family (Agnes, Mary, Joshua, Daniel, and Evans) for their unwavering support and encouragement (and for never once asking when I am going to get a 'real' job). Thanks to my dear cousins, Rhoda, Kyalo, Kezia, Mutheu, Serita, Ezra, Dennis, Kyalo, Mark, Beth and Faith, for the fabulous company, in particular for making Christmas worthwhile.

Finally, I have saved the best until last (sorry Naomi!). Thanks Naomi for keeping me sane, believing in me, for your attention, 24/7, all year round, and being there for me throughout. Here's to the next adventure!

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Publications as a result of this thesis

1. **Dishon Muloi, Melissa J Ward, Amy B Pedersen, Mark Woolhouse, Eric M Fèvre, Bram van Bunnik**. Are food animals responsible for transfer of antimicrobial-resistant *Escherichia coli* or their resistance determinants to human populations? A systematic review. Foodborne Pathogen Diseases.

2. **Dishon Muloi, Eric M Fèvre, Judy Bettridge, Robert Rono, Daniel Ong'are, James M Hassell, Maurice K Karani, Patrick Muinde, Bram van Bunnik, Alice Street, Margo Chase-Topping, Amy B Pedersen, Melissa J Ward, Mark Woolhouse**. A cross-sectional survey of practices and knowledge among antimicrobial retailers in Nairobi, Kenya. Journal of Global Health.

3. **Dishon Muloi, John Kiiru, Melissa J. Ward, James M. Hassell, Judy M. Bettridge, Timothy P. Robinson, Bram A.D. van Bunnik, Margo Chase-Topping, Gail Robertson, Amy Pedersen, Eric M. Fèvre, Mark E. J. Woolhouse, Erastus K. Kang'ethe, Samuel Kariuki.** Epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanising city. International Journal of Antimicrobial Agents

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Abbreviations

A4NH	Agriculture for Nutrition and Health
AFLP	Amplified fragment length polymorphism
AFLP	Amplified fragment length polymorphism
AIC	Akaike information criteria
AMR	Antimicrobial resistance
ANOVA	Analysis of Variance
AST	Antimicrobial susceptibility testing
BLAST	Basic Local Alignment Search Tool
CARD	Comprehensive Antibiotic Research Database
CCs	Clonal complexes
CDC	Centers for Disease Control and Prevention
CLSI	Clinical and Laboratory Standards Institute
CPD	Continuing professional development
CRE	Carbapenems-resistant *Enterobacteriaceae*
DDDs	Defined Daily Dose
E. coli	*Escherichia coli*
EGLIDE	Edinburgh Glasgow Liverpool Disease Ecology group
EMBA	Eosin methylene blue agar
EMBA	Eosin methylene blue agar
ESBLs	Extended-spectrum β-lactamase
ESEI	Ecology of Human Infectious Diseases Initiative
GEMS	Global Enteric Multi-Center Study
GLASS	Global AMR Surveillance System
GLM	Generalized linear model
GLMMs	Generalised linear mixed effects models
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HGT	Horizontal gene transfer

HICs	High income countries
ICEs	Integrated conjugative elements
IFPRI	International Food Policy Research Institute
IFRA	Institut Français de Recherche en Afrique
ILRI	International Livestock Research Institute
IQR	Inter-quantile range
IREC	Institutional Research Ethics Committee
KPC	*Klebsiella pneumoniae* carbapenemase
LMICs	Low- and middle-income countries
MDR	Multidrug resistant
MGEs	Mobile genetic elements
MIC	Minimum inhibitory concentration
ML	Maximum likelihood
MLST	Multilocus sequence typing
MMM	Modernising Medical Microbiology Oxford
MRSA	Methicillin-resistant *Staphylococcus aureus*
MST	Minimum spanning tree
MVLA	Multiple-Locus Variable number tandem repeat Analysis
NACOSTI	National Commission for Science, Technology and Innovation
NCBI	National Center for Biotechnology Information
NDM_1	New Delhi metallo-β-lactamase
NGS	Next-generation sequencing.
NMDS	Non-metric multidimensional scaling
ODK	Open Data Kit
PBP	Penicillin-binding protein
PCA	Principle component analysis
PCoA	Principal Coordinates Analysis
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PHE	Public Health England

PMQR	plasmid-mediated quinolone resistance
QRDR	Quinolone Resistance Determining Regions
SD	Standard deviations
SLV	Single locus variant
SNP	Single nucleotide polymorphism. Variation of only one base.
ST	Sequence type
TSI	Triple sugar iron agar
UrbanZoo	Urban Zoonosis Project
WGS	Whole genome sequencing
WHO	World Health Organization

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Chapter 1
Introduction to antimicrobial resistance

> Mr. X. has a sore throat. He buys some penicillin and gives himself, not enough to kill the Streptococci but enough to educate them to resist penicillin. He then infects his wife. Mrs. X gets pneumonia and is treated with penicillin. As the streptococci are now resistant to penicillin the treatment fails. Mrs. X dies.
> Who is primarily responsible for Mrs. X's death?
>
> **Alexander Fleming, 1945**

Chapter 1 Introduction to antimicrobial resistance

1.1 The global status of antimicrobial resistance

Antimicrobial resistance (AMR) is a major global public health concern (WHO, 2015b) which complicates the treatment of infections and is associated with increased morbidity and mortality of common diseases (Bush and Fisher, 2011; Davies, 2011). Since the discovery of penicillin in 1928 (Abraham and Chain, 1940), antimicrobials have played a vital role in reducing morbidity and mortality from infectious diseases; however, these healthcare gains are now under threat because of the emergence and spread of drug resistant pathogens. In particular, the emergence and global dissemination of multidrug resistant (MDR, resistant to multiple drug classes) bacteria has enormous implications for healthcare delivery and population health (Smith and Coast, 2013).

A recent synthesis of evidence indicated that an estimated 700,000 deaths globally were attributable to infections caused by antimicrobial resistant organisms, and this could reach 10 million/year by 2050 (O'Neill, 2016).In 2011, the Centers for Disease Control and Prevention (CDC) reported that AMR associated infections and complications were responsible for 23,000 deaths in the United States of America in 2013 (CDC, 2013). Similarly, in the European Union, it is estimated that the number of deaths attributable to AMR bacteria is about 25,000 each year (ECDC and EMEA Joint Working Group, 2009). Considering the sub-optimal hygiene conditions, high burden of infectious diseases, lack of AMR surveillance and the dearth of antimicrobial stewardship programs in most developing nations, the burden of AMR may be more evident in these settings. While the general consensus is that AMR burden is high in most low- and middle-income countries (LMICs), a lack of standardized and comprehensive data prevents an accurate quantification of morbidity, mortality and economic cost associated with AMR infections (Laxminarayan, 2014). However, there are some country-specific examples; in 2010, a study in Thailand suggested that 43% (19,122 of 45,209) of deaths caused by hospital-acquired

MDR bacterial infections in Thailand were excess mortality due to MDR (Lim et al., 2016), significantly higher than in high income countries (HICs).

In the last decade, the emergence and rapid global dissemination of resistance to antimicrobials considered of last resort has been reported as a serious public health concern. For example, emergence and rapid increase in carbapenems-resistant *Enterobacteriaceae* (CRE) such as *Klebsiella pneumoniae* carbapenemase (KPC) (Yigit et al., 2001) and New Delhi metallo-β-lactamase (NDM1)(Kumarasamy et al., 2010) heralded the advent of what was described as a global "antimicrobial apocalypse". Colistin had long been considered as an antimicrobial of last resort in human medicine, usually reserved to treat MDR Gram negative bacteria due to the absence of acquired resistance (Falagas and Kasiakou, 2006; Cai et al., 2012). However, in 2015, Liu *et al* reported the discovery of a transferrable plasmid mediated colistin resistance gene, mcr-1, in *E. coli* bacteria from pigs, food and humans (Liu et al., 2015). Since then, mcr-1 (and the associated plasmid(s)) has been identified in different hosts in several countries across five continents (Wang et al., 2018).

In addition to the global public health burden, AMR infections add considerable costs to already overburdened health care systems nationally and globally (Smith and Coast, 2013). Recent predictions suggest that the economic burden of AMR infections could result in a reduction of 2% to 3.5% in global gross domestic product in 2050, amounting to between 60 and 100 trillion US dollars, globally (Adeyi et al., 2017). Further, according to the same report, AMR is projected to have a significant impact on livestock production, with estimates predicting a reduction of 2.6%-7.5% in global livestock production by 2050. The relative economic costs associated with AMR infections are speculated to be higher in LMICs, and are likely to increase as resistance to second- and third-generation antimicrobials develops, leading to situations where critically ill patients need supportive care, and antimicrobials no longer have therapeutic efficacy.

1.2 Burden of AMR in Kenya

The mortality attributable to Gram negative AMR pathogens, increasingly involved in infectious diseases in humans, has been described in healthcare and community settings in differing prevalence across numerous developing nations. As elsewhere in sub-Saharan Africa, data on antimicrobial resistance burden is scant and patchy in Kenya, both from the clinic and community (WHO, 2014). There are, however, a small number of cross-sectional surveys mostly from tertiary healthcare facilities, typically found in large cities and urban areas. A study from a large private hospital in Nairobi suggest that among *E. coli* and *Klebsiella pneumoniae*, the two most frequent bacteria cultured from clinical samples, 8% were extended-spectrum β-lactamase (ESBLs) producers. This same study showed that MDR carriage was common, with susceptibility limited to carbapenems and nitrofurantoin (Maina et al., 2013). However, carbapenem resistance is also emerging, with the first report in Africa of the NDM-1 β-lactamase gene identified in *K. pneumoniae* reported in the same hospital (Poirel et al., 2011). Similarly, carbapenem resistance bacterial infections have been reported elsewhere in Kenya (Ayoyi et al., 2017; Henson et al., 2017).

Likewise, in Kenya, the burden of AMR carriage in livestock is yet to be evaluated. A study on faecal carriage of MDR *E. coli* isolates from pigs, chicken and cattle meant for slaughter at two abattoirs in Nairobi demonstrated a high prevalence of MDR carriage (Kikuvi et al., 2006). The same study demonstrated that despite banning chloramphenicol for farm animal use in Kenya, 5% of *E. coli* isolates from pigs were resistant to chloramphenicol. In another study, resistance against tetracyclines (76%) and cotrimoxazole (72%), chloramphenicol (13%) and ciprofloxacin (19%) were detected in *E. coli* isolates from chicken meant for slaughter in Nairobi (Adelaide et al., 2008).

Whilst the highlighted studies identified high carriage of AMR in a variety of bacterial pathogens, most of these studies focused on either human or livestock

populations without making comparisons of resistances between the two populations.

1.3 Antimicrobial resistance in *E. coli*

Escherichia coli is a facultative anaerobe, Gram negative rod that colonises the lower gut of humans and animals (Ingraham and Neidhardt, 1996). Although *E. coli* can be a harmless gut commensal, some pathogenic variants cause life-threatening bloodstream infections, and other common infections, such as urinary tract infections (Dobrindt, 2005). Data from the Global Enteric Multi-Center Study (GEMS) - a large case-control study investigating the burden of paediatric diarrheal disease in sub-Saharan Africa and South Asia – identified enterotoxigenic *E. coli* as a major causative agent of severe diarrhoea among children in these areas (Kotloff et al., 2013). Phylogenetic analysis suggests that *E. coli* can be grouped into seven major phylogenetic groups (A, B1, B2, C, D, E and F) known as phylogroups (Clermont et al., 2000). Human and animal commensal strains belong mostly to groups A and B1 respectively, suggesting an association between phylogenetic groups and host species. Conversely, human pathogenic strains belong to categories B2 and to a lesser extent D (Escobar-Paramo et al., 2006).

The β-lactam class of antimicrobials, particularly cephalosporins, are the main drug class used to treat *E. coli* infections. However, in the last decade, the incidence of *E. coli* infections resistant to new generation cephalosporins has increased worldwide (Coque et al., 2008a). The emergence and global spread of resistance to fluoroquinolones, carbapenems and colistin is also a threat (Collignon, 2009; Liu et al., 2015). Typically, MDR bacteria are associated with nosocomial infections. However, MDR *E. coli,* including ESBL-producing strains, have increasingly been associated with community-acquired infections. In setting such as Nairobi, where animals and humans co-habit, these AMR *E. coli* are capable of colonizing humans and animals and of flowing between them. However, the relative roles of humans and livestock in the emergence and

dissemination of AMR bacteria and their AMR determinants remain poorly understood.

1.4 Development and spread of AMR bacteria and AMR determinants

AMR itself may arise *de novo* by via mutations in chromosomal genes and spread clonally with the bacteria, or be encoded on mobile genetic elements such as plasmids that are transferred horizontally between bacterial strains or species (Blair et al., 2015).

1.4.1 Innate mechanisms of resistance

Innate resistance refers to the *de novo* development of resistance from spontaneous mutation in the bacteria thus generating a resistant phenotype. Point mutations under selection pressure within bacterial genomes have an important role in the development and evolution of antimicrobial resistance in particular pathogens (Davies and Davies, 2010). In general, such mutations resulting in AMR alter the antimicrobial action via one of the following mechanisms: (i) altering the antimicrobial target thus decreasing the affinity for the drug, (ii) a decrease in the drug uptake, (iii) activation of efflux mechanisms to expel the drug from the cell, or iv) upregulating the production of enzymes that inactivate the antimicrobial agent (e.g. erythromycin ribosomal methylase in *staphylococci*) (Figure 1.1) (Mcmanus, 1997; Piddock, 2006; Sandegren and Andersson, 2009). In *E coli*, mutational alterations in the Quinolone Resistance Determining Regions (QRDR) in the *gyrA* and *parC* genes of bacteria are recognized to be the major mechanisms through which resistance to fluoroquinolones develops (Waters and Davies, 1997).

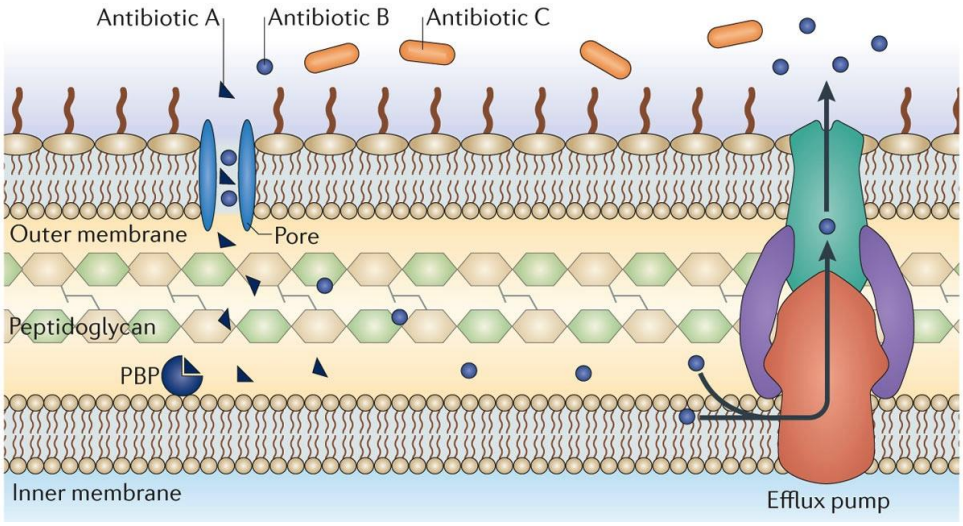

Figure 1.1 An overview of innate resistance mechanisms of β-lactam antimicrobials targeting a penicillin-binding protein (PBP). While antimicrobial A and B can enter the cell via a membrane-spanning porin protein, antimicrobial C cannot cross the outer membrane and so is unable to access the target PBP. Adapted from https://www.nature.com/articles/nrmicro3380#f1 (Blair et al., 2014).

1.4.2 Mechanisms of horizontal gene transfer

Bacteria also develop resistance to antimicrobials through the acquisition of new genetic material from other resistant organisms (Tenover, 2006; Bennett, 2008; Boerlin and Reid-Smith, 2008). This is termed horizontal gene transfer (HGT) or lateral gene transfer (Danchin, 2016), and may occur between bacterial strains of the same or different species and/or genera and include transduction, conjugation and transformation (Mcmanus, 1997). The uptake of ready-made genes from the 'mobile gene pool' enables rapid evolutionary adaptation to novel ecological niches, such as new hosts, without the reliance upon rare beneficial mutations arising from spontaneous mutation in the bacteria in the population (Jain et al., 2003).

Transformation is regarded as a parasexual process that is defined as the internalization of exogenous DNA and subsequent integration into the recipient bacterial chromosome by homologous recombination (Tenover, 2006). Unlike transduction and conjugation, transformation is entirely directed by the

recipient cell and all required proteins are encoded in the core genome (Johnston et al., 2014).

During conjugation, plasmid borne resistance genes are transferred from one bacterium to another via the *pilus,* an elongated proteinaceous appendage found on the surface of bacteria. In transduction, bacteriophage vectors transfer AMR genes between two bacteria. Both transduction and conjugation are mediated by semi-autonomous vectors: bacteriophages and conjugative elements such as conjugative plasmids, or chromosomally integrated conjugative elements (ICEs) and conjugative transposons respectively. Of the above-mentioned mobile genetic elements (MGEs), conjugative plasmids are the most significant.

Plasmids are extra chromosomal, self-replicating circular or linear fragments of DNA found in bacteria (Leplae et al., 2004). Conjugative (that is they encode the functions necessary to promote cell-to-cell DNA transfer, particularly their own transfer) or mobilisable plasmids can horizontally disseminate resistance genes among bacterial pathogens. Based on the relatively conserved regions in the plasmid genome, molecular characterisation reveals that certain plasmid incompatibility types are more clinically relevant in disseminating antimicrobial resistance genes (e.g. *IncI1, IncA/C, IncF* and *IncH12*) than others (Carattoli, 2011). Studies from numerous parts of the world have revealed the distribution of plasmids harbouring ESBL genes such as bla_{CTX}, bla_{SHV}, bla_{CMY}, and bla_{TEM} and plasmid-mediated quinolone resistance (PMQR) genes (*qnrA, qnrB,* and *qnrS*) in *E. coli* (Huddleston, 2014; von Wintersdorff et al., 2016). Of particular concern is the increasingly reported spread of plasmids harbouring resistance determinants to critically important antimicrobials such as carbapenems (Carattoli, 2013). As evident in the Liu *et al* study (Liu et al., 2015) , the *mcr1* gene, encoding colistin resistance, was initially located on a *IncI2* plasmid, but in subsequent isolates was located on *IncHI2* and *IncX4* plasmid family types, suggesting multiple pathways for horizontal dissemination (Hasman et al.,

2015). Furthermore, multiple AMR genes are often co-localized on the same plasmid, which allows for the relatively easy spread of MDR (von Wintersdorff et al., 2016).

1.5 Global and local patterns of antimicrobial usage in humans and animals

The use of antimicrobials in clinical and veterinary medicine has led to a decrease in the burden of infectious diseases in both humans and animals, and facilitated complex medical interventions such as organ transplantation (Smith and Coast, 2013). However, antimicrobial use, misuse or overuse in human and animal health exerts selective pressures on bacterial pathogens leading to development of AMR.

1.5.1 Antimicrobial usage in humans

Global consumption of human antimicrobials increased by 36% between 2000 and 2010 with India having the world's highest consumption of antimicrobials closely followed by China and the USA (Van Boeckel et al., 2014). Further, the same study noted significant increases in the consumption of monobactams, glycopeptides, cephalosporins, and fluoroquinolones. This increase was more evident amongst the BRICS countries (Brazil, Russia, India, China and South Africa) that accounted for 76% of the global increase (Figure 1. 2).

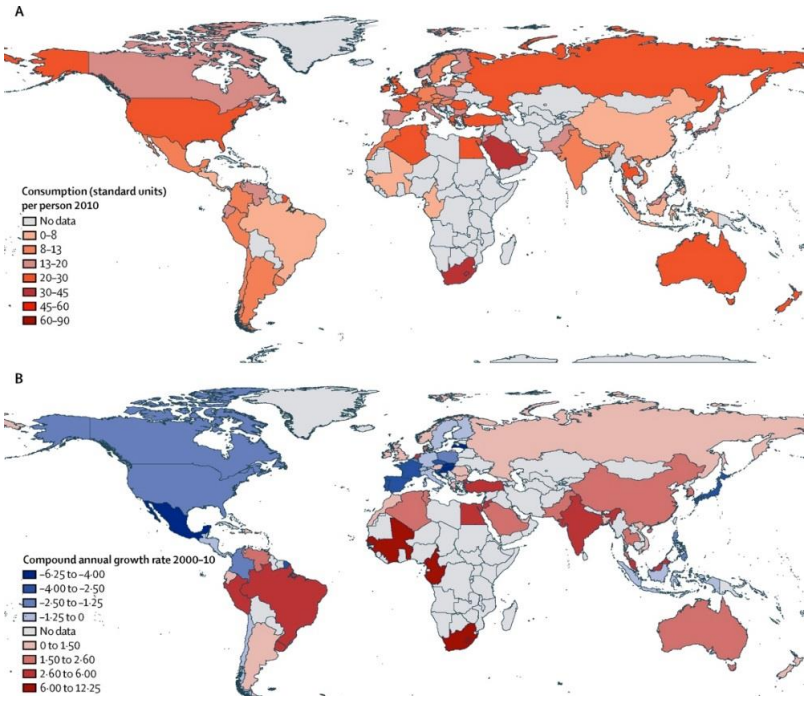

Figure 1.2 Global human consumption of antibiotics in 2010. Expressed in standard units i.e. pills, capsules per person. Source: Van Boeckel et al 2014.

In agreement with Van Boeckel et al 2014, results from a recent analysis of global antimicrobial sales data, indicate that antimicrobial consumption rates in humans increased between 2000 and 2015 (Klein et al., 2018). Further, the same study noted that while the antimicrobial consumption rates in most LMICs were lower when compared to HICs, despite higher bacterial disease burden, consumption in LMICs was rapidly converging to rates similar to HICs (Figure 1.3). This increase, the authors postulate, may partly be a result of improved access to antimicrobials because of economic development in LMICs.

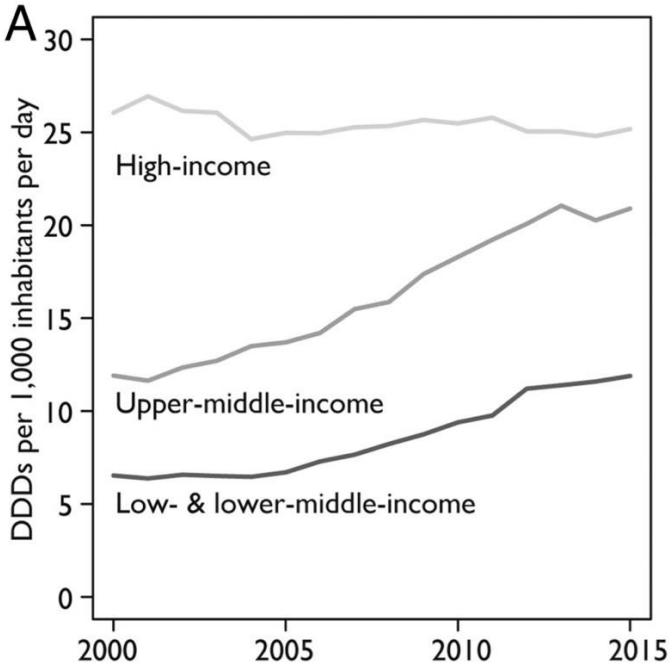

Figure 1.3. Graph showing how the global antibiotic consumption rate (DDDs per 1,000 inhabitants per day) has rapidly increased for LMICs, while remaining nearly constant for HICs. Source (Klein et al., 2018).

While there are been only handful formal data in Kenya regarding antimicrobial consumption at a national level, there have been attempts to understand prescribing and practice locally (Mitema and Kikuvi, 2004; Omulo et al., 2017; Opanga et al., 2018). One of these studies, based on antimicrobial import data, estimate that, from 1997-2001, consumption of antimicrobials in clinical medicine increased by 4%, with penicillins and fluoroquinolones being the most widely used antimicrobials (Mitema and Kikuvi, 2004). A point prevalence study in a referral hospital in Western Kenya reported that third generation cephalosporins (55%), imidazole derivatives like metronidazole (41.8%) and broad spectrum penicillins (41.8%) were the most common prescribed antimicrobials (Opanga et al., 2018).

1.5.2 Antimicrobial usage in livestock

Antimicrobials are also widely used in livestock for treatment of infections, prophylaxis and metaphylaxis (Marshall and Levy, 2011). In 2006, use of sub-therapeutic doses of antimicrobials for growth-promotion was discontinued in the European Union (Phillips, 2007), and indications are, for example in the pork and poultry sectors in Denmark, that the prevalence of AMR has decreased since then (Aarestrup et al., 2001). Livestock husbandry systems in most LMICs have a higher dependency on antimicrobials because of a more disease-prone environments and lower levels of biosecurity than in HICs (Grace, 2015; Ayukekbong et al., 2017). However, although antimicrobial overuse in intensive production is emerging in these settings, lack of access to antimicrobials among smallholders in LMICs hinders optimal livestock production (Robinson et al., 2016b).

In 2010, a study estimated the global consumption of antimicrobials in food animals at 63,151 tons and is projected to rise by 67% (105,596 tons) by 2030 (Van Boeckel et al., 2015), with the majority of that increase occurring in emerging economies where the demand for livestock products, particularly poultry and pigs, is growing rapidly (Figure 1.4).

For instance, the same study projects that, in China – the largest producer and user of antimicrobials in the world – the livestock sector could consume a third of the antimicrobials produced worldwide by 2030. These estimates suggest that antimicrobial use in food animals outranks human usage.

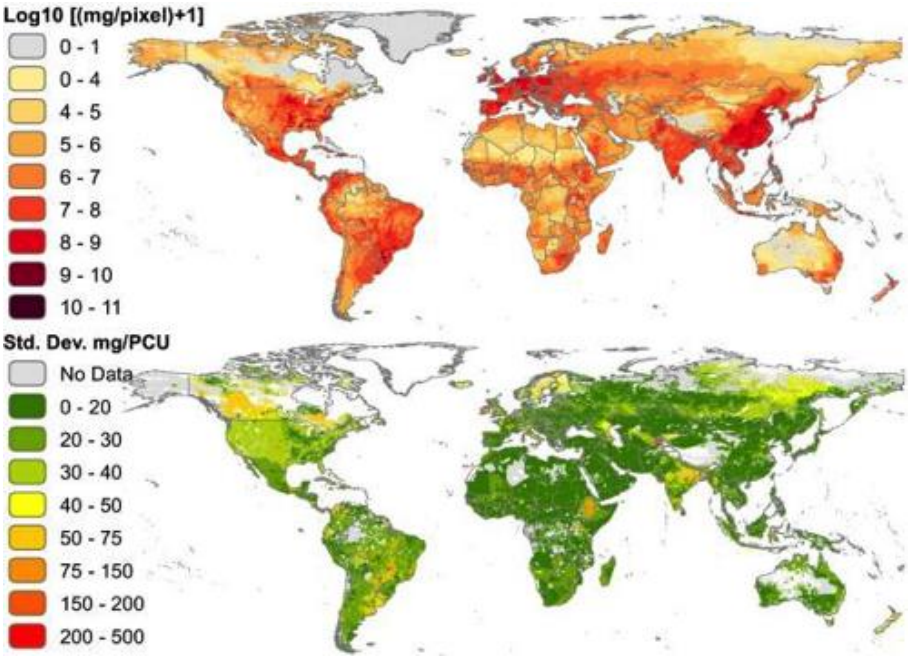

Figure 1.4. Graph showing the global antibiotic consumption rate in livestock (milligrams per 10 km2 pixels (Top) and average SD of estimates of milligrams per PCU (bottom)). Source Van Boeckel et al., 2015.

1.5.3 Surveillance of antimicrobial usage in LMICs

Monitoring antimicrobial usage – particularly for WHO-classified highest priority critically important antimicrobials – in both human and animal populations allows identification of areas in which targeted interventions hold promise of reducing drivers of resistance (Morgan et al., 2011).

Currently, most LMICs lack structures capable of quantifying consumption of antimicrobials at sufficient resolution to provide usage data by antimicrobial class, population type, purpose of usage, and route of administration, which are necessary to facilitate effective interventions to optimize use (WHO, 2018). Similarly, in these settings, the ecosystem in which human and livestock antimicrobials are produced, distributed, and consumed including the numerous possible data sources is complex and variable, and therefore makes data collection on antimicrobial use challenging. A variety of approaches are available for assessing patterns of antimicrobial use in humans and animals and include: (i) total national imports, (ii) drug sales volume from local

manufacturers and wholesalers, (iii) point prevalence data on antimicrobial consumption collected at health care or farm level (Queenan et al., 2017) (Figure 1.5). The multiplicity of methods for quantifying usage hinders comparability of antimicrobial consumption across countries, and importantly between human and animal populations.

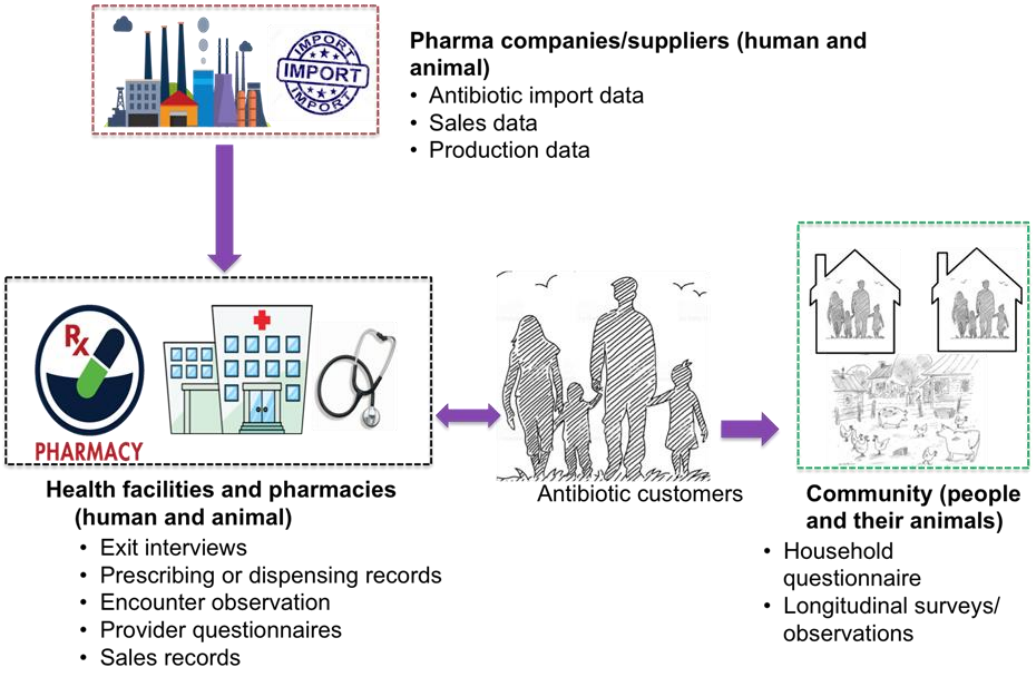

Figure 1.5. A schematic diagram indicating possible sources that can be used to obtain information on antibiotic consumption in humans and animals.

1.5.4 Antimicrobial use in food animals: risk to AMR in humans

Currently, there is no consensus regarding the interplay between antimicrobial use in food-producing animals and subsequent development of AMR in human population. In the recent past, a plethora of studies have suggested that widespread antimicrobial use in food animals might contribute to the development of resistance to antimicrobials commonly used in human medicine, especially given the overlap of antimicrobials used for these different purposes (Marshall and Levy, 2011; Landers et al., 2012). Collectively, these studies highlight the complexities in understanding the burden and transmission of AMR among populations (Woolhouse et al., 2015). A recent systematic review indicated that interventions that limit antimicrobial use in food animals reduced AMR bacteria in these animals by up to 39%, and a similar association in the studied human populations (Tang et al., 2017). Conversely, a modelling study indicated that interventions curtailing antimicrobial consumption in animals have, as a stand-alone measure, little impact on the carriage of AMR in humans (van Bunnik and Woolhouse, 2017). Evidence relating to the potential for transfer of AMR from food animals to humans will be discussed in chapter two. However, briefly, three key pathways of transfer of AMR bacteria and their AMR determinants from food animals to humans have been postulated: (i) direct acquisition of resistant pathogen from livestock, (ii) clonal transfer of a resistant pathogen to humans, followed by transmission in the human population, and (iii) horizontal transmission of AMR genes from livestock to humans (Chang et al., 2015).

1.6 Characterisation of *E. coli* and AMR

1.6.1 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) methods are used to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates (Jorgensen and Ferraro, 1998). Further, ASTs can help to identify isolates with defined resistance mechanisms of major interest to public health (for example, ESBL producers) (Pereckaite et al., 2018). Several different AST methods are available for use by clinical microbiology laboratories: broth dilution tests, antimicrobial gradient method, and disk diffusion test. Of the three methods, disk diffusion test is the widely used in the vast majority of clinical laboratories in LMICs, because it is simple and standardized (Bauer et al., 1966). In this method, the zones of growth inhibition around each of the antimicrobial disks (dispensed onto bacteria-containing agar plates) are measured to the nearest millimetre. The diameter of the zone is related to the susceptibility of an isolate and those values translated to categories of susceptible, intermediate, or resistant using the latest tables published by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2016) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Matuschek et al., 2014).

For most antimicrobials, there is good correlation between inhibition zone diameters and minimum inhibitory concentration (MIC) (Ericsson and Sherris, 1971) which is considered the 'gold standard' against which all other susceptibility testing methods, and breakpoints are assessed and set respectively (Matuschek et al., 2014). Although ASTs are an integral part of microbiology laboratory, they are time consuming (turnaround times are usually between 12 h and 48 h), as they involve regrowth of the organism in the absence and presence of the relevant antimicrobials. As such, genotypic methods are increasingly being used to identify specific AMR genes or genetic mutations using molecular or genomic (usually DNA-based, amplification-based or sequencing-based) methods. Whereas various genotypic methods are available for generating

antimicrobial susceptibility profiles in bacterial pathogens (e.g. PCR or microarrays) (Sabat et al., 2013), whole genome sequencing (WGS) yields far more information and does not require *a priori* knowledge of the resistance phenotype of the isolate (Ellington et al., 2017).

1.6.2 The impact of whole genome sequencing on microbiology

In recent decades, whole genome sequencing has become an increasingly important technology for understanding pathogen evolution, AMR surveillance, and genomic epidemiology in most modern clinical laboratories (Didelot et al., 2012). As WGS allows comparison of the genetic differences between organisms down to the resolution of a single base pair, this method has several advantages in terms of: (i) providing unprecedented level of strain discrimination including insight into the evolutionary context of the strain, (ii) identifying potential epidemiological linkages, including when other metadata that could be used to infer epidemiological links is not available, (iii) inferring geographical origins of an outbreak strains from the phylogenetic signal, and (iv) identifying of genetic elements that may result in pathogenicity (e.g. virulence factors) or AMR (e.g. AMR genes and point mutations associated with reduced susceptibility) (Baker et al., 2018b; Jenkins et al., 2019).

The advent of high throughput next-generation sequencing (NGS) technologies, including 454 pyrosequencing, Illumina and Ion Torrent sequencing, has led to large scale analyses of pathogen genomes and the costs of bacterial WGS continue to decline. Currently, the sequencing cost of a bacterial genome using NGS can be as little as $100, including sample preparation, library quality control, and sequencing (Motro and Moran-Gilad, 2017). This reflects a 400-fold reduction in costs as compared with only 8 years ago. As a result, comparative analysis of WGS is now the reference typing method used in outbreak studies in some countries; for instance, in 2015, Public Health England (PHE) adopted WGS as the molecular typing method of choice for investigating food-borne outbreaks of STEC O157:H7 in England (Dallman et al., 2015).

WGS provides the current 'gold standard' resolution for studying genetic relatedness and determining the evolutionary origins of pathogen lineages – a key element in understanding the transmission of AMR bacteria and AMR determinants between hosts. The genetic relatedness of isolates can be determined by the phylogenetic analysis of the single-nucleotide polymorphisms (SNPs), or with core genome multilocus sequence (cgMLST) (Kluytmans-van den Bergh et al., 2016). The major advantage of SNP analysis or cgMLST lies in the increased resolution than traditional molecular tools such as multilocus sequence typing (MLST) or fingerprint-based methods, like pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) (Schürch and van Schaik, 2017). Of the aforementioned methods, MLST, based on 7 housekeeping genes, is the most widely used method for determining bacterial population structures, and assigning isolates to lineages, as it is a relatively fast, accurate, and reproducible tool (Maiden et al., 1998).
A study based on genetic markers (MLST) revealed a broad genetic similarity between chicken and human *E. coli* strains, suggesting clonal transfer (Leverstein-van Hall et al., 2011); however, subsequent WGS revealed that the isolates were actually separated by 1,263 core genome SNPs (de Been et al., 2014) highlighting the potential 'added value' of WGS. When combined with phylogeographic methods, WGS yields the potential for quantitative hypothesis testing for inferring pathogen movement between host populations (Ward et al., 2014; Richardson et al., 2018). For example, a recent study, combining WGS and discrete state phylogeographic analysis, identified successful host-switching events of *Staphylococcus aureus* clones between humans and livestock hosts (Richardson et al., 2018).

In the last decade, through WGS analysis of bacterial pathogens, we have a better understanding of the range of different genes associated with AMR. Identification of these genes is important to understand how and where AMR develops and spreads across different host populations and ecological niches. Several AMR gene databases and bioinformatic tools that enable the

identification of AMR genes from whole- or partial-genome sequence data, including unannotated raw sequence assembly contigs, already exist. These AMR databases are either downloadable for use locally (for example using ARIBA (Hunt et al., 2017) or SRST2 (Inouye et al., 2014)) or are web-based solutions and include: ARG-ANNOT (Gupta et al., 2014), ResFinder (Zankari et al., 2012a), and Comprehensive Antimicrobial Research Database, CARD (McArthur et al., 2013). While these tools are invaluable for understanding resistance in bacteria, their success is hinged on comprehensiveness and quality of the AMR gene databases used – most of which are heavily biased towards human pathogens, and commonly studied bacteria (Boolchandani et al., 2019). In addition, complex resistance mechanism, such as those associated with overexpression of AMR genes (Shigemura et al., 2015) or epistatic relationships between multiple genes (Baroud et al., 2013), are challenging to identify in AMR gene databases.

While *in silico* analysis of bacterial genomes for the presence of AMR genes or point mutations known to confer resistance data is now widely accepted for public health investigations, the use of WGS to predict antimicrobial susceptibility phenotype (genotype-to-phenotype) is still an area of active development (Ellington et al., 2017). A small number of studies have assessed the feasibility of using WGS to infer AMR in *E. coli* (Stoesser et al., 2013) (Ingle et al., 2018). These studies are mostly based on screening for known AMR genes or point mutations known to confer resistance (e.g. AMR mutations associated with ciprofloxacin resistance). In one study, Stoesser *et al.* reported the overall sensitivity and specificity of the genotypic prediction when compared with standard phenotypic test for five antimicrobials (amoxicillin, co-amoxiclav, gentamycin, ciprofloxacin, ceftriaxone, ceftazidime and meropenem) as 0.99 and 0.96 respectively.

Further, WGS is central to the development of a culture-independent method of bacterial identification – metagenomics. Although yet to be implemented in

routine diagnostics, metagenomic analysis is an important tool to characterise the genomic composition, including diversity and abundance of bacterial communities thus bypassing the bottleneck imposed by single colony selection (Pehrsson et al., 2016; Munk et al., 2017). The use of metagenomic analyses in monitoring AMR in host populations has been undertaken; for example, a recent study reported that AMR resistomes in pig and poultry isolates obtained across Europe were different in abundance and composition (Munk et al., 2018).

In order to translate genomic data into actionable knowledge that can be used to improve surveillance and control of AMR, a recent review highlights the need for combining the high resolution offered by WGS with high-fidelity epidemiological metadata (EFSA, 2013). At the very least, AMR surveillance studies should identify high-risk populations (human and animal) for AMR transmission, and provide specific guidance for reducing AMR carriage.

While this technological advancement is unfolding, the uptake and utilization of the genome sequencing technologies in most LMICs is limited mainly due to the high cost of establishing and maintaining a sequencing facility and lack of skilled personnel (Helmy et al., 2016).

1.7 Research background

As elsewhere in sub-Saharan Africa, a combination of growth and rural-urban migration has led to substantial increase in the population of urban and peri-urban Nairobi, the capital city of Kenya, rising from 2m in 1999 to 3.5m in 2013 (KNBS, 2014). In urban Nairobi, people live in a continuum of urban spaces with varying population densities, with the vast majority of people living in slums, often characterised by small household areas and high population density (estimated as 28,200 people per km^2) (Joshi et al., 2011; Bird et al., 2017). This rapid population shift is associated with social disparity, poor sanitation, poor living conditions, and poses a risk to human health through the emergence and spread of infectious diseases (Yang et al., 2012). Given the high infectious disease burden in both human and animal populations, antimicrobial usage is a crutch that is used as a low-cost alternative to comprehensive hygiene and biosafety measures (in animal production) – a significant driver of AMR. To date, few studies have investigated the burden of AMR studies in LMICs, highlighting the need for further research is in such settings (Dar et al., 2016).

Livestock have been implicated as a reservoir for AMR bacteria that may spread to humans, with the keeping of livestock widely thought to be a risk factor for AMR in humans (Bélanger et al., 2011; O'Neill, 2015). However, quantitative evidence regarding the role of livestock in the emergence and transmission of AMR bacteria and their resistance determinants are lacking. Studies investigating the epidemiology of AMR have tended to focus on either human or livestock populations without making comparisons of resistances between the two populations. In particular, few studies have included urban livestock, which are increasingly important, particularly in LMIC settings (Satterthwaite et al., 2010) and may contribute to the maintenance of zoonotic bacteria and AMR in the complex urban environment.

Urban Nairobi is an ideal place to analyse the complex interaction of pathogens between and within human and livestock populations: the wealthy live

alongside the poor, livestock live alongside people, human and livestock waste is poorly disposed, and largely unregulated food systems take place in connected networks (Roesel and Grace, 2014; Alarcon et al., 2017). The close degree of mixing and contact between livestock and humans creates diverse ecological niches (or 'interfaces') (Hassell et al., 2017), which present broad opportunities for either population to act as a reservoir from which AMR bacteria or their AMR determinants could be transmitted in either direction.

This thesis focuses on the role of livestock keeping as a potentially high-risk interface for AMR transmission between humans and livestock in urban Nairobi using *E. coli* a proxy for transmission. *E. coli* is an ideal organism to study the spread of AMR in this complex environment since it is a commensal in both human and food animal populations, shares the same niche as enteric pathogens and is genetically diverse (Jaureguy et al., 2008; Tenaillon et al., 2010). The hypothesis underpinning this study is that there is an epidemiologically significant spillover of AMR bacteria and AMR determinants from livestock to human populations.

1.8 Research objectives

In this thesis, I use a combination of genomics, epidemiological and ecological methods to investigate patterns of bacterial strain sharing and AMR in *E. coli* in sympatric human and livestock populations in Nairobi, Kenya. In this respect, my thesis can be split into four broad themes:

(i) Review of existing evidence (and the nature of evidence used to support, or not support) that food animals are responsible for transfer of resistant *E. coli* and their AMR determinants to humans.

(ii) Characterising the patterns and epidemiology of AMR-*E. coli* carriage from co-habiting human and livestock populations.

(iii) Analysis of AMR gene co-occurrence patterns and the role of conjugative plasmids on the epidemiology of AMR spread.

(iv) Genetic analysis of *E. coli* isolates to elucidate patterns of bacterial relatedness and strain sharing as a proxy for transmission potential.

(v) A cross-sectional study to investigate the patterns of antimicrobial use in humans and livestock in urban Nairobi and importantly, and help in explaining the phenotypic and genotypic AMR patterns identified in human and livestock populations in this thesis.

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Chapter 2
Materials and methods

> The term epidemiology is often preceded by an adjective, making reference to the topic of its application. One of the most iconic and dramatic of these adjectival descriptors is *shoe-leather*, raising the image of an on-the-ground investigation racing to find a solution to a deadly epidemic.
>
> **Jonathan M. Samet, 2010**
>
> **(Quoting Alexander D. Langmuir, 1910–1993)**

Chapter 2 Materials and methods

2.1 Introduction

This chapter provides a detailed overview of methodological elements that are common to chapters throughout the thesis, including study design, data collection in the field, laboratory procedures, certain analytical approaches, and the statistical frameworks that underpin them. Additional methods relating to analysis of data that are unique to particular analyses, are outlined in detail in the relevant chapters.

2.2 Ethics statement

The collection of data adhered to the legal requirements of the International Livestock Research Institute (ILRI). Ethical approval for human sampling and data collection was obtained from the ILRI Institutional Research Ethics Committee (ILRI IREC) ILRI-IACUC2015/09. ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federal wide Assurance (FWA) for the Protection of Human Subjects in the United States of America. Livestock samples were obtained under the approval of the ILRI Institutional Animal Care and Use Committee (reference ILR-IACUC2015/18). The antimicrobial usage survey was conducted under approval of project reference ILRI-IREC2017-35.

2.3 Study design

This thesis research is part the UrbanZoo project (http://www.zoonotic-diseases.org/project/urban-zoo-project/) a Medical Research Council-funded project that aimed to utilise a landscape genetics approach to understand the movement and sharing of pathogens in a major developing city. A significant component of the UrbanZoo project was the '99 household project' which focused on sampling of households across socio-economic strata of Nairobi to investigate the role of informal livestock keeping practices as a route of zoonotic disease emergence in humans.

As such, a cross-sectional study targeting sympatric human and livestock populations in Nairobi, Kenya was carried out from August 2015 to October 2016 with the aim of maximising the spatial distribution and diversity of livestock keeping practices across the city while capturing socio-economic diversity and population distribution. Geospatial mapping data produced by Institut Français de Recherche en Afrique (IFRA) was used to identify 17 classes of residential neighbourhood in Nairobi based on different land-use patterns (e.g. residential, industrial, institutional) and physical landscape attributes (such as tree cover, plot size, amount of gated space, roofing type, presence of agriculture) (Ledant et al., 2011). The choice of the 17 different classes of residential neighbourhoods was premised on the assumption that neighbourhoods with similar physical characteristics are also similar in terms of other qualities, such as socio-economic status, and service provision. The classification was validated with 817 household questionnaires across the city.

Subsequently, the 17 classes of neighbourhoods were ranked by average income and merged into seven wealth groups (Table 2.1). Administrative boundaries were overlaid on maps of each wealth group, which yielded 70 sub-locations. To maximise geographical spread across the city, while ensuring equal representation across the different wealth categories, a population-weighted approach was used to decide how many sub-locations should be chosen from each wealth group (Table 2.1). Thereafter, thirty three households were chosen based on: a) selection of slum and peripheral rural areas with high livestock densities, in which previous project activities had been carried out (8 sub-locations); b) selection of one sub-location to represent each remaining neighbourhood class (15 sub-locations); c) selection of 10 further sub-locations to make up the target number for each wealth group, attempting to maximise both spatial distribution, socio-economic diversity, and proportion of population belonging to the dominant class (Figure 2.1).

Table 2.1. Distribution of the seven wealth groups used by the Urban Zoo Project, and the number of sub-locations with a dominant wealth group identified and selected across the city. Reprinted with permission (Bettridge et al., 2017).

	Characteristics of physical neighbourhood classes identified by IFRA study				**Urban Zoo Project re-classification**		
code	**Tree cover**	**Defining characteristics**	**Neighbourhood description [a]**	**Average monthly income per capita [b]**	**Wealth group**	**Possible sub-locations**	**Targeted sub-locations**
A	> 13.5%	Detached housing with intense tree cover	Detached housing on very large plots *(>3000 m²)*	39890	1	8	3
B			Detached housing on large plots *(400 - 3000 m²)*	22462	2	8	4
C	> 13.5%	Attached and semi-detached housing	Attached housing on medium plots *(<400 m²)* with important tree cover	22084	2		
D	3-13.5%	Apartment building	Apartment buildings *with gated space*	22084	2		
E	3-13.5%	Attached and semi-detached housing	Higher standing row houses *(plot size > 190 m²)*	13352	3	5	3
F			Lower standing row houses *(plot size < 190 m²)*	6153	4	3	3
G	<3%	Roof cover >50% tiles	Lower standing *apartment buildings*	6153	4		
H			New areas of dense *single housing* development	3855	5	9	5
J	<3%	Roof cover > 40% concrete	High density multi-storey buildings	3855	5		
K	3- 13.5%	Apartment building	Apartment buildings *with open access*	3855	5		
L	3- 13.5%	Peripheral areas	Peripheral areas *(mainly residential)*	3855	5		
M			Peripheral areas with rural component *(presence of agriculture)*	2165	6	24	11
N	3- 13.5%	Collective housing	Community housing *with gated space*	2165	6		
P			Community housing *with open access*	2165	6		
Q	<3%	Roof cover >85% corrugated iron sheets	New areas of low quality housing *(built-up area <37%)*	2165	6		
R			High density planned low quality housing *(built-up area <37% AND public space >20%)*	2165	6		
S			High density unplanned low quality housing (slums) *(built-up area <37% AND public space <20%)*	1301	7	13	4

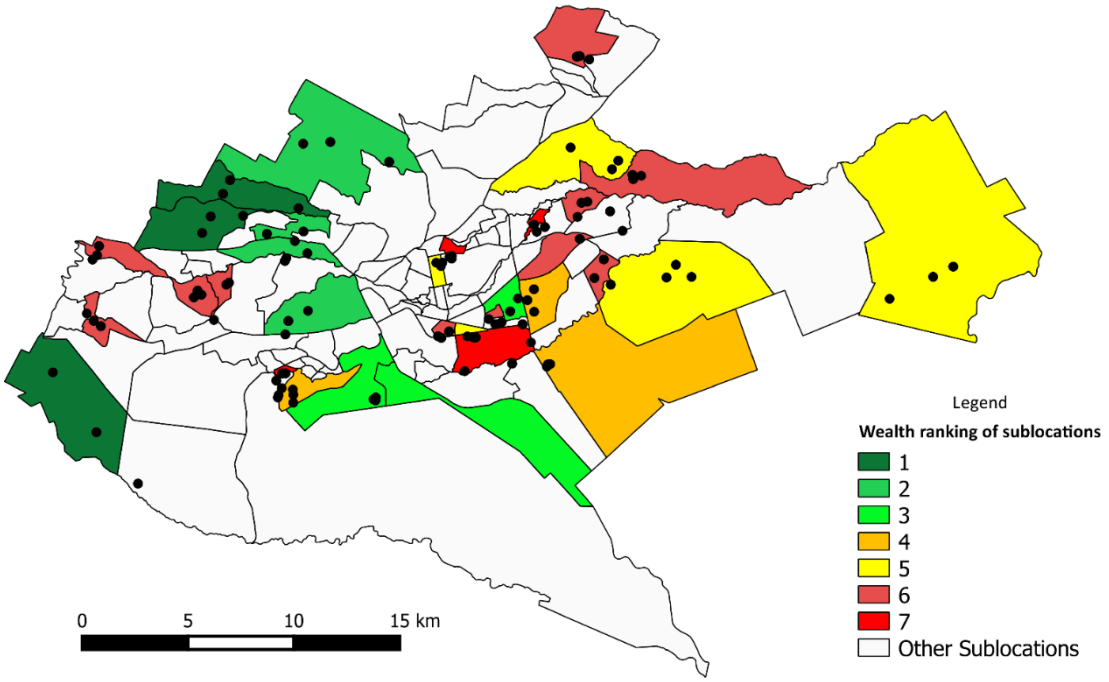

Figure 2.1. Map of the 99 households (black dots) and 33 sublocations (coloured by wealth category; 1 – wealthy, 7 – poor) in Nairobi city selected for inclusion in the study.

For each sublocation, three geographical points were selected at random within the dominant housing type, comprising of: two livestock keeping and one non-livestock keeping household. A total of 99 households, 66 of which kept livestock were visited. Livestock keeping households had to meet strict inclusion criteria of: (i) keeping small livestock only (small ruminants - goats/sheep, small monogastrics - poultry/rabbits), and (ii) large livestock (large ruminants (cattle), large monogastrics (pigs), with or without small livestock (Table 2.2). To ensure an equal sample of both cattle and pig-keeping households, the combination of livestock keeping households represented in each sublocation was randomised, and had to consist of either large ruminant and small monogastric, or large monogastric and small ruminant species. For sublocations in which households keeping large ruminant or large monogastric species were absent, a replacement household keeping either small monogastic or small ruminant species was recruited.

Table 2.2. Criteria used in defining livestock keeping households

	Large ruminant	**Large monogastric**	**Small ruminant**	**Small monogastric**
Necessary and sufficient	Cattle	Pigs	Goats/sheep	Poultry or rabbits
Optional	Any other species	Any other species	Pigs, poultry or rabbits	Cattle, sheep or goats
Exclusion	None	None	Cattle	Pigs

Within the sublocations, local administrative leaders assisted in recruitment, which was carried out a few days before the sampling date. The three pre-selected geographical points were identified on the ground, and the nearest three households that met the inclusion criteria identified.

In a household, human members were defined as those who either slept on the premises or (for staff) spend at least 8 hours a day on the household, and interacted regularly with the main household members in way to facilitate exchange of bacterial pathogens. Such interactions included; sharing food prepared on the premises, regularly handling food, animals, animal manure or contact with human excrement (for example nannies looking after young children). Conversely, humans who lived on the household (e.g. tenants) but who had had separate cooking facilities and did not contact or share livestock or livestock products belonging to the core household were excluded.

2.4 Data Collection

In each household, the household head/owner (or a nominated member) completed a questionnaire, detailing livestock ownership (e.g. abundance of livestock species), management practices (e.g. manure disposal practices), household composition (e.g. number of occupants), and socio-economic variables.

Thereafter, following a written informed consent, every human member of the household was invited to contribute a faecal sample and answer questionnaires on: their age and gender, food consumption and medical history. Rectal swabs were obtained from (up to 20) livestock species present in the household (ensuring that all species were represented). Project clinicians, and veterinarians collected human and livestock faecal samples respectively. Questionnaires and data associated with samples was recorded using Open Data Kit (ODK) Collect software, on electronic tablets, and uploaded to databases held on servers at ILRI. Human and animal faecal samples were collected and transported on ice to one of two laboratories (University of Nairobi or Kenya Medical Research Institute) within 5 hours of collection. For details of questionnaire data, see appendix Tables A1-4.

2.5 Laboratory work

Samples were enriched in buffered peptone water for 24 hours, and thereafter plated onto eosin methylene blue agar (EMBA) and incubated for 24 hours at 37°c. One colony from each plate was selected and sub-cultured for a further 24 hours on a second round of EMBA. Consequently, one purified colony from each plate was selected at random (hereafter referred to as an 'isolate'), and confirmed as *E. coli* by biochemical testing, using triple sugar iron agar (TSI), Simmon's citrate agar, and motility-indole-lysine media. *E. coli* isolates preserved by freezing (-20°c then -80°c) in 15% (v/v) Glycerol. Between the two laboratories, laboratory protocols were standardised and a project

microbiologist was responsible for ensuring that these standards were maintained.

2.5.1 DNA extraction and whole genome sequencing

Bacterial genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Life Technologies) according to the manufacturer's instructions. The DNA concentration and purity was measured using NanoDrop 2000c spectrophotometer and the Qubit double-stranded DNA HS Assay Kits (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation was conducted according to the Illumina protocol and sequenced on an Illumina HiSeq2500 (Illumina, San Diego, CA, USA) at the Wellcome Trust Centre for Human Genetics.

2.5.2 Sequencing quality control

For quality control purposes and to check for any variation arising at the laboratory or sequencing stage (Robasky et al., 2014), a validated reference [CFT073 strain; GenBank: AE014075.1; NCBI RefSeq NC_004431] and a duplicate of a randomly chosen sample from each DNA plate were included at specified locations on each sequencing plate. Furthermore, cross-plate sequencing replicates were also performed across sequencing batches.

2.6 Bioinformatic analysis

Whole genome sequencing (WGS) was carried out at the Wellcome Trust Centre for Human Genetics on the Illumina HiSeq 2500 platform. 150 base-pair paired-end reads were generated and short-read WGS data were pre-processed using an automated protocol developed by the Modernising Medical Microbiology Oxford (MMM) Group to: (i) perform standard quality control checks using fastQC (Andrews, 2010) with default settings; (ii) trim reads to remove remnant adaptor sequences using bbduk, part of the BBTools package (Bushnell, 2014), (parameters: minoverlap=12, k=19, mink=12, hdist=1, ktrim=r) and (iii) perform a Kraken (Wood and Salzberg, 2014) speciation analysis

against an in-house database downloaded from the NCBI sequence read archive (www.ncbi.nlm.nih.gov/sra/), with an automated step for removal of contaminant (non-bacterial) reads. De novo assembly of *E. coli* isolates was performed using SPAdes v3.64 (Bankevich et al., 2012) (parameters: --careful, -t 1, --phred-offset 33). Samples deemed as non-*E. coli* on the basis of the speciation analysis were excluded from further analysis. Potentially mixed *E. coli* samples were identified as those with an unusually large assembly size (greater than 6 megabases (Mb)) and were removed from the dataset.

Further downstream processing of *E. coli* isolates including antimicrobial susceptibility testing, *in silico* MLST analysis, identification of antimicrobial resistance determinants and further genetic analysis are explained in the relevant chapters.

2.7 Statistical Analysis

This section provides, in brief, an overview of the statistical analyses that were carried out in this thesis. Other methods with specific details, where used, are described in the relevant chapters. Throughout this thesis, *a priori* hypotheses, in which biological understanding of the underlying system was taken into consideration, were used to guide the statistical analysis.

2.7.1 Data exploration

Following recommendations by (Zuur et al., 2010), in this thesis I routinely investigated: (i) collinearity between explanatory variables, (ii) influence of outliers in the response or explanatory variables, and (iii) zero inflation (excess number of zeros). Outlier detection is based on the assumption of an underlying known distribution of the data, which is assumed to be identically and independently distributed. Box plots were used to visualise the median and the spread of the data. Where outliers in explanatory variables were present (e.g. count of livestock), log base 10 transformations were applied to the variable in question.

To test for collinearity between explanatory covariates, for each model, relationships between all sets of explanatory covariates were assessed using multi-panel pairwise scatterplots, Pearson correlation coefficients, and variance inflation factors. Where an excess frequency of zeros (zero inflation) was present in the response variables, zero inflated generalised linear mixed effects models (GLMMs) were used.

2.7.2 Parametric and non-parametric tests

Parametric tests assume underlying statistical distributions (e.g. normal distribution) in the data and hypothesis testing is based on the assumptions made. Conversely, nonparametric tests do not rely on any distribution (Fay and Proschan, 2010).

The following parametric techniques were used for exploratory data analysis and to test for associations:

(i) Descriptive statistics, to provide summaries about the means and variability (standard deviations: SD and standard errors: SE) of the variables of interest.

(ii) The Pearson's correlation coefficient (r), to estimate the strength of association between pairs of continuous variables.

The non-parametric statistics used included:

(i) The Mann-Whitney U Test, used to test the null hypothesis that two samples come from the same population (i.e. have the same median)

(ii) The Chi-square (χ^2) test, used to test for associations between independent categorical variables and the Fisher's exact test, used for small sample size test for associations between categorical variables.

Post-hoc comparisons were performed on categorical variables to identify which sub-groups differed significantly between each other. Throughout the thesis,

where stated, a Bonferroni's test was used to adjust for multiple pairwise comparisons.

2.7.3 Generalised Linear Mixed Models

Generalized linear mixed models (GLMMs) are an extension of linear mixed models to allow response variables from different distributions, such as binary responses, and to include both fixed and random effects (Zuur et al., 2009). GLMMs are used throughout this thesis to investigate the presence of a relationship between variables of interest, while accounting for potential non-independence introduced by a hierarchical sampling design.

The simplest form of a GLMM model (in matrix notation) may be written as:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Z\gamma} + \varepsilon$$

where *y* is the n x 1 vector of the response variable, β is a p x 1 vector of the fixed-effects regression coefficients, Z is the N×q design matrix for the *q* random effects (the random complement to the fixed X), γ is a q×1 vector of the random effects (the random complement to the fixed β), and ε is a N×1 column vector of the residuals.

Deciding the model structure for GLMMs was based on knowledge about the relation between Y and X. Where the response variable was a dataset of counts (e.g. number of AMR genes), Poisson GLMMs were used. For binary outcomes (e.g. presence or absence of an AMR gene), binomial GLMMs were employed. When modelling count data with an excess of zero counts, zero inflated Poisson GLMMs were used. Zero inflated Poisson GLMMs model count data that exhibit a bimodal distribution due to both excess zeros and positive counts, thus allowing for a large number of zero cases (e.g. a large number of pan-susceptible isolates) without compromising the model.

Random effects were included in models throughout this thesis, unless otherwise stated, to account for the three tiers of nestedness of our sampling design; isolates nested within households (n=99), household-level data grouped into triplets by sublocation (n=33), and sublocations nested in wealth categories (n=7) (Figure 2.2).

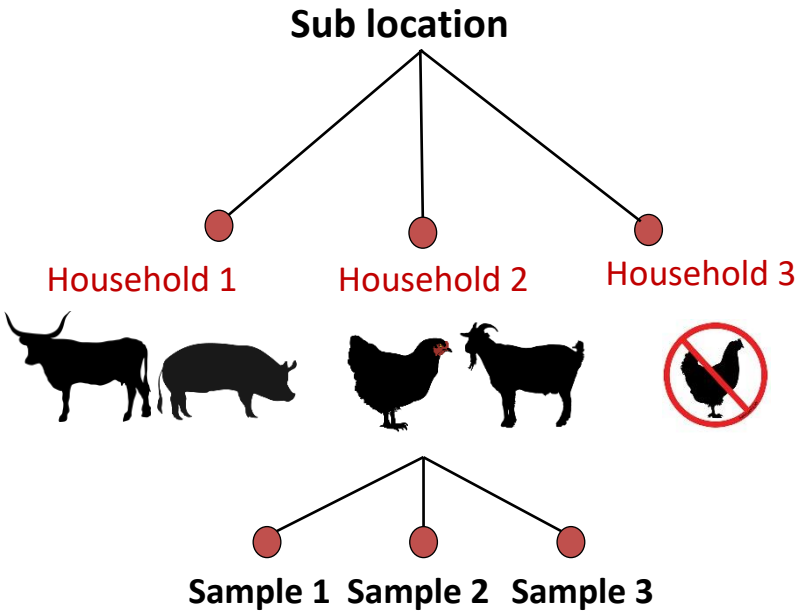

Figure 2.2. Hierarchical sampling design of the 99 households project. Samples are nested within households, whilst triplets of households are nested within sublocations.

Model building was based on biologically plausible hypotheses. Best fitting models were estimated by backward exclusion of non-significant terms (p=0.05), using Akaike information criteria (AIC), and log-likelihood ratio tests. From global models, backward selection procedure for model selection was used to sequentially drop variables that give the highest AIC values and largest p-value. Model fit was checked by plotting the residuals versus fitted values and normal probability plots. GLMMs were implemented in the R packages 'lme4' (Bates et al., 2014) and 'glmmTMB' (Magnusson et al., 2017) (for zero inflated models).

2.7.4 Multivariate statistics

Multivariate statistical analyses are often used in community ecology to summarise high-dimensional data, test hypotheses involving multiple response variables, and examine relationships between large sets of variables (Härdle and Simar, 2007). In this thesis, two multivariate analytic methods were used; non-metric multidimensional scaling (NMDS) and principle component analysis (PCA).

NMDS was used to evaluated whether AMR gene communities in human and livestock (and the different livestock groups) populations had different compositions (beta-diversity). Compositional dissimilarity of AMR gene assemblages between isolates was estimated with the Bray–Curtis dissimilarity index (Bray and Curtis, 1957), and pair-wise dissimilarities between isolates were analysed by a non-metric multidimensional scaling (NMDS) plot. NMDS was performed with the metaMDS function of the R vegan package (Oksanen et al., 2015). Kruskal's Stress, defined as goodness of fit between similarity rankings and ordination distance rankings, was calculated and visualized in a Shepard plot (a plot showing the relationship between the actual dissimilarities between objects (from the original dissimilarity matrix) and the ordination distances).

PCA is a reduction method that transforms high-dimensional data (mostly highly correlated) into fewer dimensions 'principal components' that explain much of the variance and represent unobserved characteristics of the population (Lever et al., 2017). In this thesis (chapter 7), PCA was used to generate a knowledge index as a composite measure of respondent's knowledge about antimicrobial resistance. The first principal component explains the largest proportion of the total variance and was used as the knowledge index. As such, results of the first principal component were used as inputs to regression analyses to investigate the possible influence of type of drug store (human or veterinary), clinical training (present or absent), education level

(high or low), and range of antimicrobials sold in the drug store (proxy for store size) on the knowledge index.

2.8 Production of maps

Maps of Nairobi were generated in QGIS Development Team (2018). QGIS Geographic Information System. Open Source Geospatial Foundation Project (http://qgis.osgeo.org).

Chapter 3

Are Food Animals Responsible for Transfer of Antimicrobial-Resistant *Escherichia coli* or Their Resistance Determinants to Human Populations? A Systematic Review

Work in this chapter has been published in Foodborne Pathogens Disease, and a copy of the publication is included in Appendix I

> When you can measure what you are speaking about and express it in numbers, you know something about it; but when you cannot express it in numbers your knowledge about it is of a meagre and unsatisfactory kind
>
> **Lord Kevin, 1883**

Chapter 3 Are food animals responsible for transfer of antimicrobial-resistant *Escherichia coli* or their resistance determinants to human populations? A systematic review

3.1 Abstract

The role of farm animals in the emergence and dissemination of both AMR bacteria and their resistance determinants to humans is poorly understood and controversial. Here I systematically reviewed the current evidence that food animals are responsible for transfer of AMR to humans.

I searched PubMed, Web of Science and EMBASE for literature published between 1940 and 2016. My results show that eight studies (18%) suggested evidence of transmission of AMR from food animals to humans, 25 studies (56%) suggested transmission between animals and humans with no direction specified and 12 studies (26%) did not support transmission. Quality of evidence was variable among the included studies; one study (2%) used high resolution typing tools, 36 (80%) used intermediate resolution typing tools, 6 (13%) relied on low resolution typing tools, and 2 (5%) based conclusions on co-occurrence of resistance. Whilst some studies suggested to provide evidence that transmission of AMR from food animals to humans may occur, robust conclusions on the directionality of transmission cannot be drawn due to limitations in study methodologies. My findings highlight the need to combine high resolution genomic data analysis with systematically collected epidemiological evidence to reconstruct patterns of AMR transmission between food animals and humans.

3.2 Introduction

At present, the role of farm animals in the emergence and dissemination of both AMR bacteria and their resistance determinants to humans is poorly understood and controversial (Marshall and Levy, 2011; Woolhouse et al., 2015). Various studies have suggested that AMR bacteria and their AMR determinants can be transmitted from food animals to humans via direct contact and/or through animal products (Howells and Joynson, 1975; Aminov and Mackie, 2007; Jakobsen et al., 2010; Overdevest et al., 2011; Kluytmans et al., 2013; Voets et al., 2013). However, most of these studies have relied heavily on traditional microbiology and molecular tools, such as PFGE and MLST. These tools may not have sufficient discriminatory power to provide evidence of the transmission (or not) of resistant bacteria and their AMR determinants and, importantly, to infer the direction of the transmission (de Been et al., 2014; Woolhouse et al., 2015). Evidence from a recent systematic review suggests that a proportion of human cephalosporin resistant *E. coli* clones, often associated with human disease, originate from food animals through food products (Lazarus et al., 2015) (though these products could have been contaminated elsewhere in the production chain (Wooldridge, 2012)).

Evidence either supporting or refuting the claim that dissemination of AMR bacteria or their resistance determinants from food animals to humans is occurring will be key to the development of effective policies on antimicrobial stewardship and infection control for both human and animal health. To address this knowledge gap, I performed a systematic review to i) explore the current evidence that food animals are of the source of resistant *E. coli* and their AMR determinants in humans, ii) examine and summarise the kinds of evidence used to support, or not support, transfer of resistant *E. coli* and their AMR determinants to humans and iii) make recommendations for future studies to address this question. *E. coli* is found in both human and food animal populations (Neidhardt et al., 1996), and has recently been categorised as one of the priority pathogens that pose the greatest threat to human health due to

widespread AMR (WHO, 2017b). It is for these reasons that, when considering transmission between hosts, I chose to focus on *E. coli*.

3.3 Methods

3.3.1 Data sources and search strategy

A systematic literature search according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (Liberati et al., 2009) was performed. Searches were carried out in multiple electronic databases: PubMed, Web of Science and EMBASE for research articles published between 1940 and 2016; and Scopus for research articles published between 1960 and 2016 without geographical and language restriction. An initial and subsequent keyword searches with various combinations of search terms: *Escherichia coli,* AMR terminologies, human, and food animal descriptors was performed (see Table A2 in Appendix A).

3.3.2 Selection criteria and data extraction

Articles were included if they comprised an original research published in a peer reviewed journal, and investigated transmission of resistant *E. coli* and/or AMR determinants between humans and food animals. Articles were excluded if: (i) they reported only agents other than *E. coli;* (ii) they studied non-food animals; (iii) they focused exclusively on food animals or humans without any overlap between the two populations and/or (iv) they focused exclusively on food of animal origin. Article searches and screening were performed by considering article titles and abstracts for inclusion according to the search criteria. Data extraction from studies was performed by one author (DMM) and independently checked by another author (BvB) using a customised checklist.

3.3.3 Data analysis

For all included studies I categorised the direction of AMR transmission according to the authors' conclusions: i) studies suggesting to provide evidence of transmission from food animals to humans with direction specified; ii) studies suggesting to provide evidence of transmission from humans to food

animals with direction specified; iii) studies suggesting overlap indicating the possibility of between-host AMR transmission, with no direction specified; and iv) studies suggesting no evidence of transmission in either direction.

The quality of evidence was assessed using a customised Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (Godfray et al., 2013). Each paper was matched to the following categories: i) high resolution typing: studies using whole genome sequencing (WGS) and phylogenetic analysis; ii) intermediate resolution typing: studies carrying out genetic characterisation through molecular tools such as MLST; iii) low resolution typing: studies using tools such as PFGE; or iv) co-occurrence of resistances: studies comparing AMR phenotypes between the two populations.

Additionally, I assessed the methodological quality of the papers included in the review by adapting a standardised quality assessment (Centre for Reviews Dissemination, 2009). Each paper was evaluated based on two items aimed at assessing potential biases including: study design (active, passive) and spatiotemporal matching (no matching, temporal matching only, spatial matching only, and both temporal and spatial matching).

Because of heterogeneity of the studies (regarding typing tools, antimicrobials investigated and quality of evidence) I did not perform a meta-analysis. However, I used Fisher's exact tests using R package 'stats' (R Core Team, 2013) to describe associations between direction of transmission, selection bias variables and nature of transmission (clonal, determinant or both). I considered $p<0.05$ to be statistically significant.

3.4 Results

3.4.1 Description of included studies

Of the 5662 distinct articles retrieved, 256 studies were reviewed (Figure 3.1); and 45 studies met all inclusion criteria (Table A1 in Appendix A).

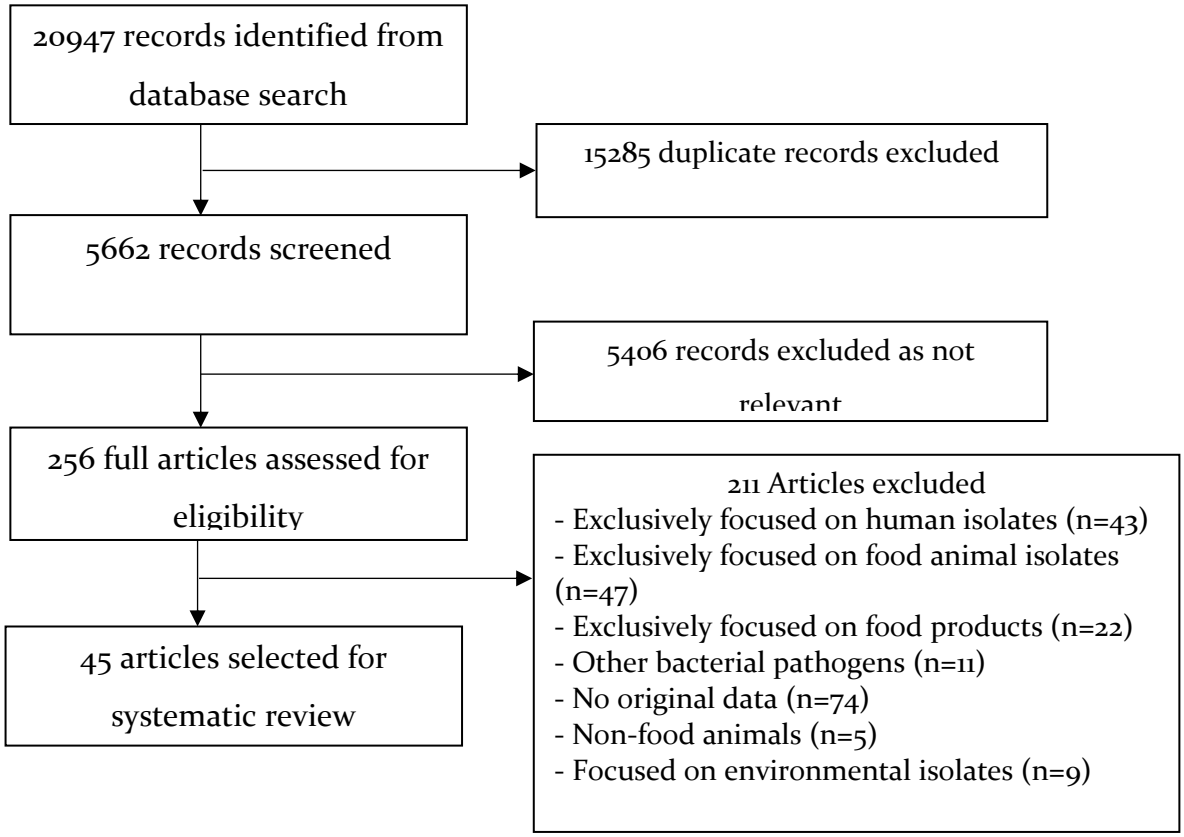

Figure 3.1. Flow diagram showing the selection of the studies for inclusion.

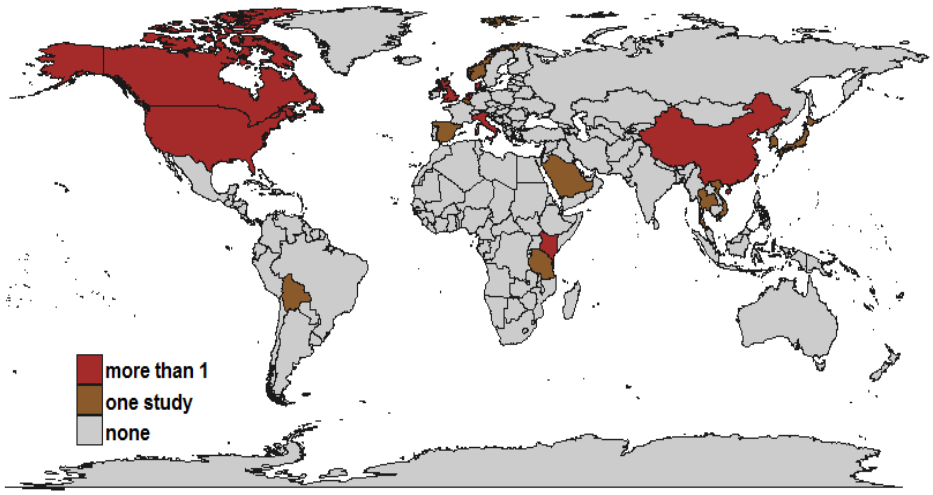

Figure 3.2. Geographic distribution of included studies. Different colours show the number of papers from each country. The map was created using several R packages (ggplot2 (Wickham et al., 2013), mapdata (Becker and Wilks, 2016), maps (Becker and Wilks, 2017), and ggmap (Kahle and Wickham, 2013)) in R version 3.4.1. The shapefile with borders of countries is freely available from the Natural Earth data set (http://www.naturalearthdata.com/).

Although the studies span five decades, there has been an increasing number of studies on this subject in recent years; with 56% of the studies published since 2010 (Table A1 in Appendix A). 22 studies (49%) had both temporal and spatial matching for human and food animal sampling, while seven (16%) had temporal matching only, and 16 (35%) were not temporally or spatially matched. There were no statistical associations between whether direction of transmission was inferred and study design or spatiotemporal matching.

Studies in this review reported different livestock species, either alone or in combination with other species. Of the eight studies that suggested transfer of AMR from food animals to humans, seven studies were based on poultry isolates and one study on pig isolates (Table A2 in Appendix A). Amongst the studies, 13 antimicrobial classes were reported, either alone or in combination with other classes (Table A3 in Appendix A).

Overall, eight studies (18%) suggested to have data to support transfer of AMR bacteria and/or their AMR determinants from food animals to humans (Levy,

1978; Al-Ghamdi et al., 1999; van den Bogaard et al., 2001; Hammerum et al., 2006; Johnson et al., 2006; Leverstein-van Hall et al., 2011; Giufre et al., 2012; Dierikx et al., 2013), while 25 studies (56%) presented data showing overlap of AMR bacteria and AMR determinants between food animals and humans, indicating the possibility of between-host AMR transmission but with no direction specified (Jorgensen, 1983; Oppegaard et al., 2001; Winokur et al., 2001; Ho et al., 2009; Moodley and Guardabassi, 2009; Mulvey et al., 2009; Smet et al., 2009; Zhang et al., 2009; Ho et al., 2010; Jakobsen et al., 2010; Zhao et al., 2010; Deng et al., 2011; Jakobsen et al., 2011; Vieira et al., 2011; Stokes et al., 2012; Ciccozzi et al., 2013; Hu et al., 2013; de Been et al., 2014; Hammerum et al., 2014; Valentin et al., 2014; Dahms et al., 2015; Dohmen et al., 2015; Huijbers et al., 2015b; Lupindu et al., 2015; Tseng et al., 2015), and 12 studies (26%) did not suggest to find evidence supporting transmission between food animals and humans (Kariuki et al., 1997; Kariuki et al., 1999; Maynard et al., 2004; Kang et al., 2005; Phongpaichit et al., 2007; Graziani et al., 2009; Schwaiger et al., 2010; Xia et al., 2010; Johnson et al., 2012; Riccobono et al., 2012; Jakobsen et al., 2015; Ueda et al., 2015). No study in this review suggested to provide evidence for AMR transmission from humans to food animals (Figure 3.3).

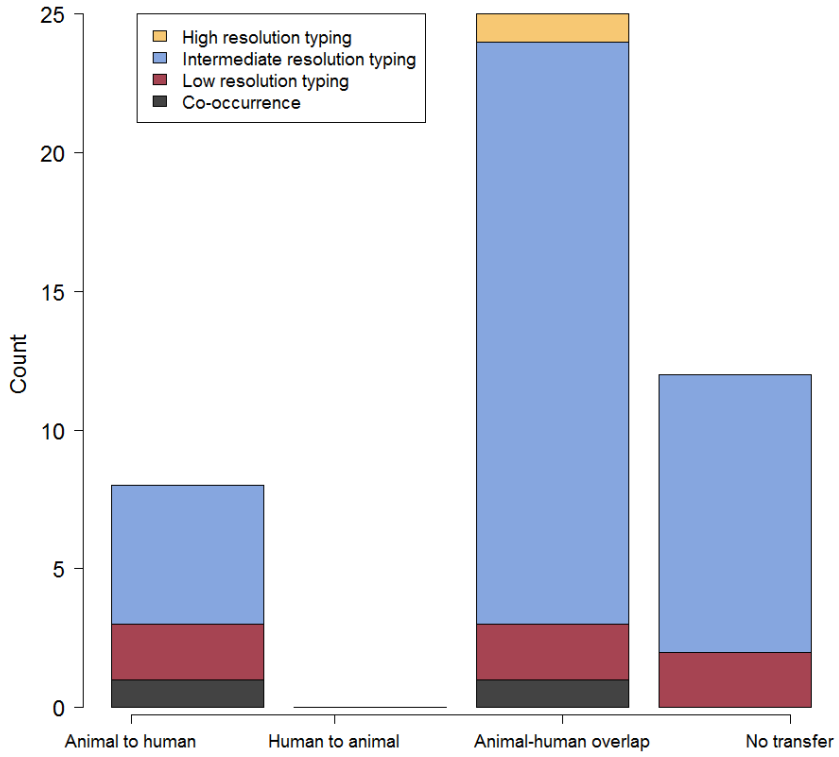

Figure 3.3. Nature of evidence

Only one study (2%) based its conclusion regarding transmission on high resolution typing tools, 36 studies (80%) on intermediate resolution typing tools, six (13%) on low resolution typing tools, and two (5%) on co-occurrence of resistances (Figure 3.3). Overall, 18 (40%) studies based their conclusion on transmission of AMR determinants, nine (20%) on transmission of AMR bacteria and 18 (40%) transmission of AMR bacteria together with AMR determinants (Table A4 in Appendix A). There were no statistical association between whether direction of transmission was inferred and the nature of transmission (p=0.33).

3.4.2 Studies suggesting to provide evidence of transmission of AMR from food animals to humans with direction specified

Three studies suggested to find evidence for transfer of AMR bacteria from food animals to humans, two of which concluded there is transfer of resistant clones from poultry to humans (Al-Ghamdi et al., 1999; van den Bogaard et al., 2001). In addition to overlapping clonal patterns, one study reported that human and chicken isolates were resistant to spectinomycin, an antimicrobial mostly used in veterinary medicine (Al-Ghamdi et al., 1999). Similarly, one study (van den Bogaard et al., 2001) reported a higher prevalence of ciprofloxacin resistance among food animal isolates compared to human isolates.

One study found identical ciprofloxacin resistant isolates in chickens and humans, which they concluded was suggestive of food animal to human AMR transmission (Johnson et al., 2006). Two studies suggested to find evidence for horizontal transfer of AMR determinants from food animals to humans (Hammerum et al., 2006; Dierikx et al., 2013). One study found that clonally unrelated poultry and human isolates shared ESBL/*AmpC* genes located on identical plasmid families (Dierikx et al., 2013). Another study found that sulphonamide resistant isolates from pigs and healthy humans shared *sul1* and *sul2* genes (Hammerum et al., 2006).

Three studies suggested to support transmission of both AMR bacteria and their AMR determinants from food animals to humans. Two studies found similar sequence types, plasmid families and ESBL genes in *E. coli* isolates sourced from poultry and human patients (Leverstein-van Hall et al., 2011; Giufre et al., 2012). A further study reported an increase in tetracycline resistant *E. coli* in humans in contact with tetracycline fed chickens and, therefore, suggested that chickens were a reservoir of AMR bacteria and plasmids for humans (Levy, 1978).

I found that studies suggesting to provide evidence of transmission of AMR from food animals to humans did not have distinct features compared to those suggesting overlap of resistance, with regard to study methodologies, food

animal species, typing tools or antimicrobials tested. For most of these it is unclear why they suggested evidence of directional transmission when 25 broadly similar studies suggested only overlap of resistance.

3.4.3 Studies suggesting overlap indicating the possibility of between-host AMR transmission, with no direction specified

Four studies suggested there was evidence of overlap of resistant *E. coli* between humans and food animals. One of these studies found human and avian sequence types associated with MDR clustered together in a Bayesian phylogenetic tree (Ciccozzi et al., 2013). Another study found indistinguishable PFGE patterns of ampicillin and tetracycline resistant isolates in cattle and humans (Lupindu et al., 2015). A cluster analysis of *E. coli* phylogroups found that human, pig and chicken isolates clustered together (Jakobsen et al., 2010). One extensive ecological study reported a significant correlation between the prevalence of AMR in human and livestock isolates, for both cephalosporins and fluoroquinolones(Vieira et al., 2011).

Thirteen studies suggested there was evidence of overlap of AMR determinants in human and food animal isolates. Of the 13 studies, one study using WGS and plasmid reconstruction found that clonally unrelated human and poultry isolates carried ESBL genes encoded on genetically identical plasmids (de Been et al., 2014). Eleven studies found that unrelated human and food animal isolates shared identical AMR genes, integrons and plasmids (Oppegaard et al., 2001; Winokur et al., 2001; Ho et al., 2009; Moodley and Guardabassi, 2009; Mulvey et al., 2009; Smet et al., 2009; Zhang et al., 2009; Ho et al., 2010; Stokes et al., 2012; Huijbers et al., 2015b; Tseng et al., 2015). One study identified identical plasmids encoding chloramphenicol resistance in unrelated human and food animal isolates (Jorgensen, 1983).

Eight studies suggested there was evidence of overlap of resistant *E. coli* and AMR determinants, with five of these finding that clonally related human and food animal isolates harboured similar ESBL gene types and plasmid types (Hu et al., 2013; Hammerum et al., 2014; Valentin et al., 2014; Dahms et al., 2015;

Dohmen et al., 2015). Likewise, two studies found that clonally related human and food animal isolates carried similar fluoroquinolone AMR genes (Zhao et al., 2010; Deng et al., 2011). In one study, cluster analysis of AMR gene profiles and *E. coli* pathotypes showed that human and food animal isolates clustered together (Jakobsen et al., 2011).

3.4.4 Studies suggesting no evidence of transmission of AMR between humans and food animals

Two studies found no evidence for transfer of resistant clones, with one of these studies finding that human and avian ciprofloxacin resistant *E. coli* strains had distinct phylogenetic compositions (Graziani et al., 2009). Likewise, a PFGE analysis of MDR *E. coli* isolates from sympatric children and chickens found that the isolates were source specific (Kariuki et al., 1999).

Three studies reported no evidence for transfer of AMR determinants between food animals and humans with one of these studies reporting that human and porcine isolates had different distribution patterns of sulfonamide and tetracycline resistance genes (Schwaiger et al., 2010). Two studies (Kariuki et al., 1997; Phongpaichit et al., 2007) reported that human and food animal MDR isolates had distinct plasmids and integrons.

Seven studies reported no evidence for transmission of bacterial clones together with AMR determinants between food animals and humans. These studies showed that human and food animal isolates belonged to different phylogenetic groups, and had different AMR genes and plasmid profiles (Maynard et al., 2004; Kang et al., 2005; Xia et al., 2010; Johnson et al., 2012; Riccobono et al., 2012; Jakobsen et al., 2015; Ueda et al., 2015).

3.5 Discussion

I performed a systematic review to explore the evidence that food animals are responsible for the transfer of AMR *E. coli* and their AMR determinants to humans. Some studies in this review suggested to provide evidence for the transfer of AMR from and between food animals and humans, while a larger number did not suggest to provide evidence of transmission in either direction. In addition to the differing nature of methods used to infer direction, studies in this review differed in sampling methodologies and antimicrobials tested. These differences may have affected the conclusions made regarding the epidemiological connection between food animals and humans.

Much of the evidence regarding transfer of AMR was based on the demonstration that AMR *E. coli* clones and AMR determinants were indistinguishable in both food animal and human isolates. However, the demonstration of overlapping patterns should be interpreted with care as the direction of transmission is difficult to infer, and co-colonisation from a shared source is also possible. Demonstrating the direction of transmission and thus the epidemiological history of pathogens and their determinants requires a quantitative description of relatedness, including phylogenetic analysis (Grad and Lipsitch, 2014).

Molecular techniques, such as MLST and PCR, used in most studies in this review, are limited in resolution (Didelot et al., 2014). In one study, *E. coli* isolates were considered genetically indistinguishable based on MLST suggesting clonal transfer (Leverstein-van Hall et al., 2011); however, subsequent WGS revealed that the isolates were genetically distinct (de Been et al., 2014), highlighting the need for sequencing the entire genome, rather than only a few loci. WGS provides the current 'gold standard' resolution for studying genetic relatedness, but as it is a technology that has only recently become routinely available it was used in just one study in this review. Future studies in this area could benefit from combining phylogeographic methods with WGS, which

yields the potential for quantitative hypothesis testing for inferring pathogen movement between host populations (De Maio et al., 2015; Woolhouse et al., 2015).

Just over half of the studies in this review did not consider spatiotemporal relationships between human and food animal isolates, a fundamental requirement for investigating transmission (Singer et al., 2006). Future research on the directionality of transmission will benefit from designing studies in which epidemiologically linked human and food animal populations are systematically sampled, preferably longitudinally (Woolhouse et al., 2015). Moreover, there is considerable diversity within both human populations (i.e. healthy individuals vs. hospitalised patients) and food animals (i.e. free range vs. intensive farming) and the specific population considered may impact their exposure to diverse groups of bacteria, thus I recommend that future studies investigating transmission of AMR between humans and food animals clearly clarify the sub-populations studied. In addition, inclusion of detailed data on antimicrobial usage in these populations should be considered.

None of the included studies provided a detailed overview of antimicrobial usage in either human or food animal populations, or association between antimicrobial usage and subsequent development of AMR. A recent systematic review has indicated that interventions that limit antimicrobial use in food animals are associated with a reduction of AMR development in humans (Tang et al., 2017), and therefore further research is warranted to explore this complex association.

Although transfer of AMR from humans to food animals is likely (Barber, 2001; Wooldridge, 2012), none of the studies in this review suggested to find evidence to support transmission from humans to animals. In many instances, responsibility for the burden of AMR has been placed on food animals (Barber, 2001; Woolhouse et al., 2015; Mendelson et al., 2017), and thus study bias may exist in terms of source attribution. Therefore, more research is needed to

provide evidence for this potential route of transfer and, importantly, the relative magnitude of that spread.

Akin to the studies in this review, most AMR studies focus on a single bacterial type; however rapid dissemination of AMR determinants frequently occurs between bacterial species, making it hard to track infection source (Sheppard et al., 2016). Tracking these determinants, frequently located on plasmids, using traditional molecular techniques may be limited. Using long read sequencing technologies such as Pacbio can overcome this by accurately generating plasmid structures (Orlek et al., 2017).

This systematic review excluded studies focusing on transmission of resistant bacteria and/or their AMR determinants through food animal-sourced food products. However, I acknowledge the potentially significant role played by food products of food animal origin in dissemination of AMR as reported in a recent systematic review (Lazarus et al., 2015).

I have highlighted studies which suggest to provide evidence for transfer of resistant *E. coli* and their AMR determinants from food animals to humans. However, differences in study methodologies, such as lack of spatiotemporal overlap in sample collection, and the quality of typing tools used, suggest that whereas transmission may occur, the evidence used to support the hypothesis is rarely compelling. The underlying problem is that demonstrating similarity or identity of AMR bacteria and/or AMR resistance determinants does not, by itself, provide information on directionality of transfer; this could be in either direction, or both, or neither but from a different source. Information on differential prevalence of resistance, and consumption of antimicrobials, in the two populations may make stronger inference possible, but these data are rarely available.

Taken together, by combining genomic data analysis and epidemiological approaches it may be possible to reconstruct the complex transmission

dynamics of resistant bacteria and their AMR determinants between human and food animal populations. Although we still have some way to go before a truly comprehensive integration of data – differential antimicrobial usage data, detailed denominator data, information about the origin of the samples, human - food animal contact data, and pathogen sequence data – is available, disentangling and quantifying transmission of resistant bacteria and their AMR determinants between humans and food animals may still be an attainable goal.

Chapter 4

Epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanising city

Work in this chapter has been published in International Journal of Antimicrobial Agents (in press), and a copy of the publication is included in Appendix I.

> In microbiology the roles of mutation and selection in evolution are coming to be better understood through the use of bacterial cultures of mutant strains.
>
> **Edward Lawrie Tatum, 1909-1975**

Chapter 4 Epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanising city

4.1 Abstract

There are substantial limitations in our understanding of the distribution of AMR in humans and livestock in LMICs. Here, I present the results of an epidemiological study examining patterns of AMR in *E. coli* isolates circulating in sympatric human (n=321) and livestock (n=633) samples from 99 households across Nairobi, Kenya. *E. coli* isolates were tested for susceptibility to 13 antimicrobial drugs representing 9 antimicrobial classes.

Overall, 47.6% and 21.1% of isolates displayed resistance to ≥ 3 and ≥5 antimicrobial classes respectively. Human isolates showed significantly higher proportions of resistance to sulfonamides, trimethoprim, aminoglycosides and penicillins compared to livestock ($p<0.01$), while poultry isolates were more commonly resistant to tetracyclines ($p=0.01$) compared to humans. The most common co-resistant phenotype observed was to tetracyclines, streptomycin and trimethoprim (30.5%). At the household level, AMR carriage in humans was associated with human density ($p<0.01$) and the presence of livestock manure ($p=0.03$), but livestock keeping on its own had no influence on human AMR carriage ($p>0.05$).

My findings revealed a high prevalence of AMR *E. coli* circulating in healthy humans and livestock in Nairobi, with no evidence to suggest that keeping livestock as a sole risk factor significantly contributed to the burden of AMR in humans, although the presence of livestock waste was significant. These results provide an understanding of the broader epidemiology of AMR in complex, and interconnected urban environments.

4.2 Introduction

Livestock have been implicated as a reservoir for AMR bacteria that may spread to humans, with the keeping of livestock widely believed to be a risk factor for AMR in humans (Bélanger et al., 2011; O'Neill, 2015). However, quantitative evidence describing the role of livestock in the emergence and transmission of AMR bacteria to human populations is lacking (Muloi et al., 2018), particularly in low- and middle-income countries (LMICs) (Dar et al., 2016). In the absence of routine surveillance of AMR in most LMICs, understanding the epidemiology of AMR is key to developing effective strategies targeting a reduction in the emergence and spread of resistance in the future.

To date, studies investigating the epidemiology of AMR have tended to focus on either human or livestock populations without making comparisons of resistances between the two populations. A recent systematic review (Muloi et al., 2018) of studies investigating the link of AMR *E. coli* between humans and livestock found only 22 studies of spatiotemporally-related isolates from human and livestock populations, just six of which were conducted in LMICs. Notably, none of these studies considered urban livestock, which are increasingly important, particularly in LMIC settings (Satterthwaite et al., 2010) and may contribute to the maintenance of zoonotic bacteria and AMR in the complex urban environment (Hassell et al., 2017).

This study focuses on the role of livestock keeping as a potentially high-risk urban interface for AMR transmission between humans and livestock in urban Nairobi. Nairobi is a rapidly growing city where livestock are commonly kept within household compounds, bringing them into close contact with people. *E. coli* is an ideal organism to study the spread of AMR in this complex environment since it is a ubiquitous commensal in both livestock and humans, but with a wide range of resistance phenotypes.

Here, I report the first study characterising the patterns and epidemiology of antimicrobial resistant *E. coli* from co-habiting human and livestock populations in a low resource urban setting. At the scale of individual households, I explore the role of livestock as risk factors for AMR carriage in humans, hence providing insight into the pathways of AMR transfer.

4.3 Methods

Details on study design, sample collection and bacterial isolation are presented in chapter 2.

4.3.1 Antimicrobial susceptibility testing (AST)

Antimicrobial susceptibility testing for 13 antimicrobials – ampicillin (10 μg/ml), amoxicillin-clavulanic acid (30 μg/ml), cefepime (30 μg/ml), cefotaxime (30 μg/ml), ceftazidime (30μg/ml), chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), gentamicin (10 μg/ml), nalidixic acid (30 μg/ml), streptomycin (25 μg/ml), sulfamethoxazole (30 μg/ml), tetracycline (30 μg/ml), and trimethoprim (2.5 μg/ml) – that are frequently used in either/both veterinary and/or human medicine in Kenya was carried out using the Kirby-Bauer disc diffusion method (Oxoid Ltd., Basingstoke, United Kingdom). Standardised protocols were used, in which antimicrobial discs were dispensed onto bacteria-containing agar plates and incubated for a maximum of 18 hours at 35°C. *E. coli* ATCC 25922 was used as a quality control of the susceptibility tests.

Clinical and Laboratory Standards Institute interpretive criteria for *Enterobacteriaceae* (CLSI, 2016) were used to determine breakpoints for classifying isolates as either susceptible ('susceptible' or 'intermediate') or non-susceptible ('resistant') for eleven of the 13 drugs. For tetracycline and trimethoprim, isolates were classified into resistant or susceptible because examination of the distributions of the zones of inhibition showed populations of isolates with distinct phenotypic resistance patterns (Table B1 in Appendix B). To describe MDR patterns, the overall resistance profile was calculated by

combining the resistance phenotype to each individual class, hereafter referred to as AMR carriage (defined as the total number of antimicrobial classes to which an isolate was phenotypically resistant).

4.3.2 Statistical analysis

The distribution of resistance phenotypes between hosts was calculated using Chi-squared tests (humans and livestock), and an one way ANOVA (human vs different livestock groups). Tukey's multiple-comparison test was performed *post-hoc* for pairwise comparisons between groups, and P values of <0.05 were considered significant.

GLMMs implemented in R package 'lme4' (Bates et al., 2014), with count of resistances per isolate as the dependent variable were used to test whether AMR carriage differed between host groups. To investigate the co-occurrence of AMR phenotypes, a pairwise co-occurrence matrix (presence and absence) of the phenotypes was constructed using polycor package (Fox, 2016) in R and the co-occurrence relationships visualized using corrplot (Wei et al., 2017) in R. A correlation between two AMR phenotypes was considered statistically significant if the P-value (adjusted for multiple testing using Bonferroni correction) was <0.05.

To investigate finer scale household-level risk factors for AMR carriage in humans, I fitted a Poisson-distributed GLMM, with the counts of resistance phenotypes as the response variable. Model explanatory variables were human density (count of people in a household as a function of household area) and types of livestock kept in the household (small livestock only, large livestock with or without small livestock, and no livestock). Additionally, for households that kept livestock, a separate Poisson-distributed GLMM was fitted to investigate the effect of human density and manure disposal practises (manure disposed in the household compound or outside) on human AMR carriage. Separate models were fitted for the most prevalent AMR phenotypes (tetracyclines, aminoglycosides, sulfonamides, penicillins, and trimethoprim).

To account for the nested (or hierarchical) nature of the sampling design household site (n=99), sublocation (n=33) and wealth category (n=7) were included as random factors.

4.4 Results

A total of 954 isolates composed of 321 human and 633 livestock *E. coli* isolates in Nairobi, Kenya, were analysed. The number of isolates obtained from each source is presented in Table 4.1.

Table 4.1. Number of human and livestock isolates collected from the 99 households from Nairobi, Kenya (2015-2016). Livestock isolates are broken down by source.

Source		Number of isolates	% of isolates
Human		321	33.7
Livestock:			
	Poultry	345	36.2
	Bovine	64	6.7
	Goat	132	13.8
	Pig	51	5.3
	Rabbit	41	4.3

4.4.1 Patterns of antimicrobial resistance in humans and livestock

The most common resistance phenotypes (>40% of resistant isolates) were those against sulfonamides, trimethoprim, tetracyclines, and aminoglycosides. A smaller percentage of isolates (<10%) were resistant to amoxicillin/clavulanic acid, cephalosporins, phenicols, and fluoroquinolones (Table 4.2 and Figure 4.1). The distribution of resistance to the individual drugs tested is given in Table s2.

When analysed by host, proportions of AMR-*E. coli* against each of the individual antimicrobial classes, except against cephalosporins, were higher in human isolates than those of animal origin. Of 321 human isolates, >40% were resistant to sulfonamides, trimethoprim, aminoglycosides, and tetracyclines. Of 633 livestock isolates, >40% of isolates were resistant to sulfonamides, tetracyclines and trimethoprim. For both human and livestock isolates, <10% of

isolates were resistant to phenicols, fluoroquinolones, cephalosporins and β-lactams. Resistance to penicillins, aminoglycosides, sulfonamides and trimethoprim was significantly more common in humans than in livestock ($p<0.01$, Chi-squared test; Table 4.2, Figure 4.1a).

The prevalence of resistance to penicillins, tetracycline, aminoglycoside, sulfonamides and trimethoprim varied significantly between humans and livestock stratified by taxonomic groups (poultry, pigs, rabbits, bovines and goats; Tukey's post hoc test). Humans were more likely to carry *E. coli* resistant to penicillins, aminoglycoside, sulfonamides and trimethoprim than all species of livestock ($p<0.05$, one-way ANOVA with Tukey's multiple-comparison test). Conversely, poultry were more likely to carry isolates resistant to tetracyclines than humans (Figure 4.1b, Figure B1 in Appendix B).

Table 4.2. Percentages of *E. coli* isolates resistant to different antimicrobial classes classified by host type (human or livestock). Numbers show percentages of isolates classified as resistant based on the zone of inhibition. Categorical interpretation is based on breakpoints used as described in the methods section. NS=Not Significant.

Antimicrobial category	**Overall (n=954)**	**Human (n=321)**	**Livestock (n=633)**	**Adj. p value**
Sulfonamides	58.2	66	54.2	0.005
Aminoglycosides	37.1	47.7	31.8	<0.001
Trimethoprim	47.3	56.1	42.8	0.001
Tetracyclines	45.7	45.5	45.8	NS
Penicillins	30.2	40.8	24.8	<0.001
β-lactam (co-amoxiclav)	1.5	2.5	0.95	NS
Phenicols	4.0	6.5	2.69	NS
Cephalosporins	3.8	2.8	4.27	NS
Fluoroquinolones	6.8	9.7	5.37	NS

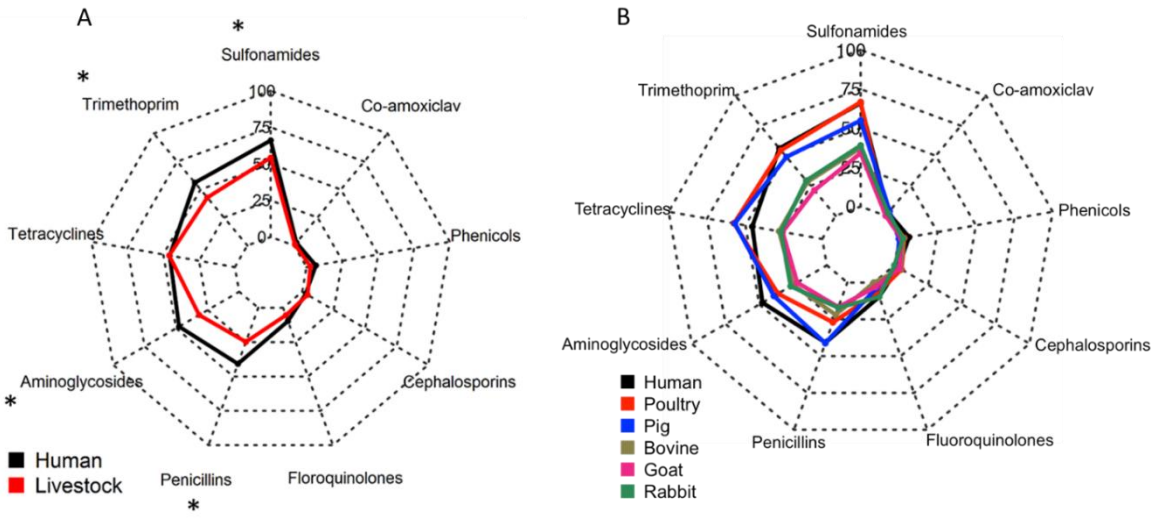

Figure 4.1 Radar charts showing percentages of E. coli isolates resistant to 9 antibiotic classes. a) human (n=321) and livestock (n=633), and b) human and the different livestock species' (poultry, pig, bovine, goat, rabbit). (Asterisks denote significant differences between carriage of this particular resistance phenotype in livestock and humans).

Overall, 284 (29.7%) isolates were susceptible to all 13 antimicrobials tested (nine antimicrobial classes). The proportion of pan-susceptible isolates was significantly higher among livestock isolates (n=217/633, 34.3%) than in human isolates (n=67/321, 20.9%) ($p<0.01$, Chi-squared test). Of the 217 pan-susceptible livestock isolates, 22% of poultry isolates (n=76), 51.6% of bovine isolates (n=33), 33.3% of pig isolates (n=17), 54.6% of goat isolates (n=72), and 46.3% of rabbit isolates (n=19) were pan-susceptible. Across both human and livestock isolates, 404 (47.6%) and 201 (21.1%) isolates were resistant to ≥ three and five antimicrobial classes respectively. Eight isolates (0.8%) showed resistance to ≥7 antimicrobial classes tested; five (1.6%) from humans and three (0.9%) from poultry (Figure 4.2).

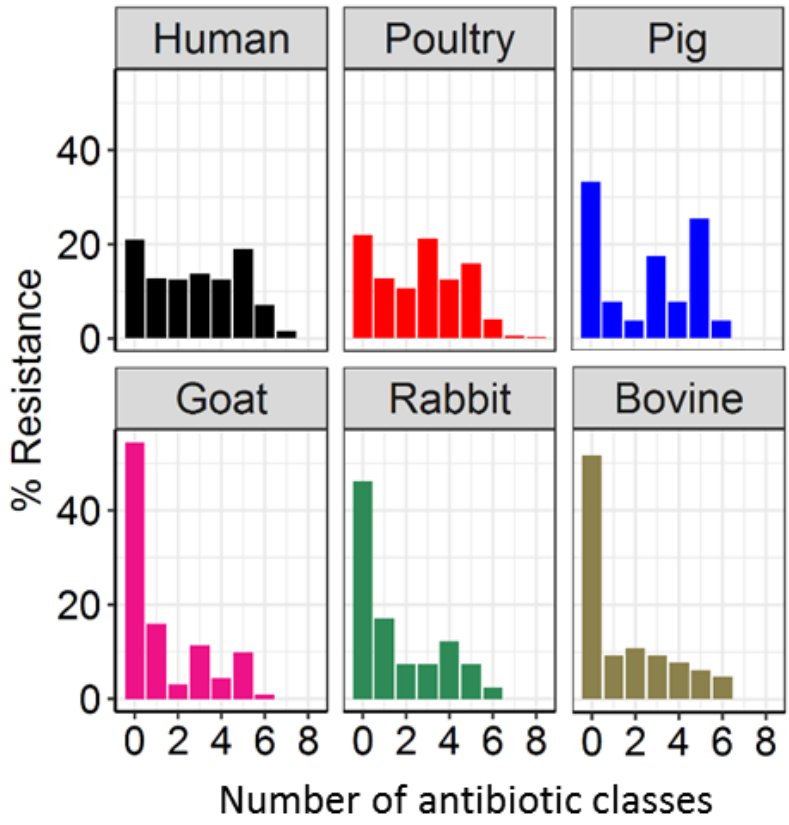

Figure 4.2 Distribution of MDR patterns among *E.coli* isolates obtained from humans (n=321), poultry (n=345), pigs (n=51), bovines (n=64), goats (n=132), and rabbits (n=41), in Nairobi, Kenya.

AMR carriage (i.e. the total number of antimicrobial classes an isolate is resistant to) was significantly higher in humans than in livestock (OR=1.14, p<0.01, GLMM). However when studied in more detail, AMR carriage patterns in human isolates was similar to those from pigs and poultry (p>0.05) but significantly higher than those from bovines, goats and rabbits (p<0.05) (Table 4.3, Figure 4.2).

Table 4.3. Results of a Poisson generalise linear mixed model examining the likelihood of AMR carriage within different host groups. Human is used as the reference level. NS =Not significant.

	No of isolates	**Estimate**	**Standard error**	**P value**
Human	321	Reference	Reference	Reference
Livestock	633	-0.13	0.16	<0.01
Bovine	64	-0.28	0.14	0.03
Poultry	345	-0.08	0.05	NS
Pigs	51	0.08	0.11	NS
Rabbits	41	-0.37	0.16	0.02
Goats	132	-0.48	0.11	<0.01

Examination of the similarity of *E. coli* antibiograms from human and livestock isolates revealed 84 distinct profiles: 30 in livestock, 19 in humans and 35 common to both (Table B4 in Appendix B). Using a co-occurrence analysis based on a statistically significant ($p<0.05$) correlation coefficient ($\rho>0.5$) I identified a tetracycline-sulfonamide-trimethoprim cluster (Figure 4.3). This co-resistance was identified in 340 isolates (30.5%): 115 (35.8%) humans and 225 (35.5%) livestock – 156 (45.2%) poultry, 24 (47.1%) pigs, 9 (22.0%) rabbits, 14 (21.9%) bovines, and 22 (16.7%) goats. There were no significant differences in the distribution of this profile between human and the other host groups (Chi-squared test; $p > 0.05$). Further, denoting multi-resistance, this cluster was commonly associated with resistance to aminoglycoside and penicillins.

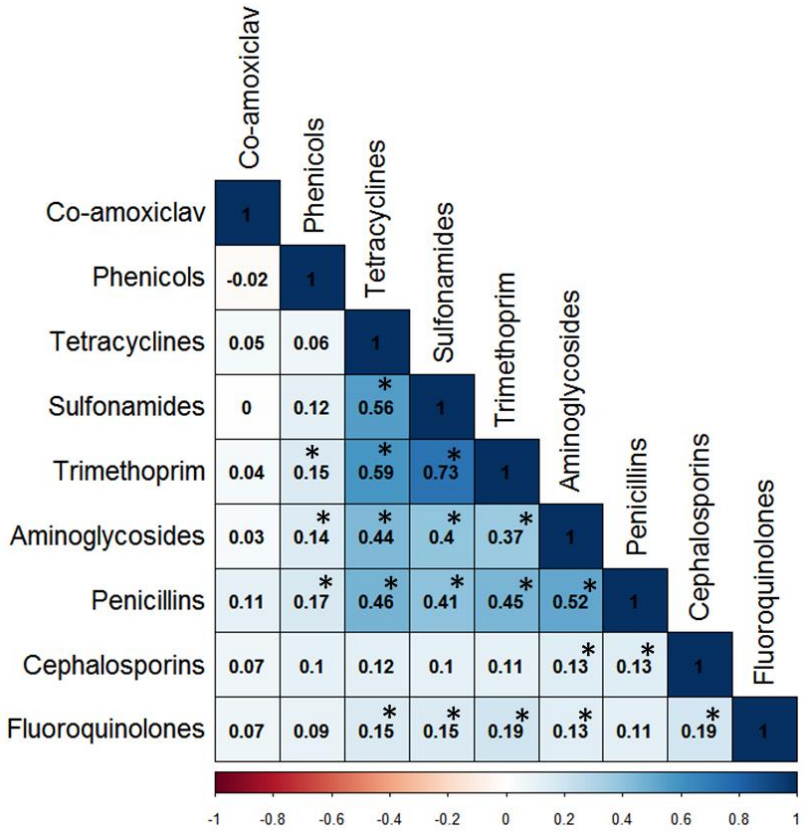

Figure 4.3 Heat map representing correlations among AMR phenotypes across human (n=321) and livestock (n=633) E. coli isolates. The boldness of the colour represents the strength of the relationship between phenotypes, with stronger correlations having bolder colours. Numbers within boxes represent correlation coefficient (r) values. * indicates statistically significant correlations ($p<0.05$). The scale bar at the bottom indicates whether the correlation between phenotypes is positive (closer to 1, dark blue) or negative (closer to –1, dark red).

4.4.2 Antimicrobial resistance exchange between humans and livestock at the household level

In any given household, I found no evidence that the presence of livestock increased risk of human AMR carriage ($p>0.05$, GLMM) (Table 4.4). However, human AMR carriage increased with human density (OR=1.26, p=0.003, 95% CI [1.08-1.47], GLMM) (Figure 4.4). The impact of livestock keeping on human AMR carriage was potentially influenced by disposal practices of animal manure: keeping manure inside the household perimeter, compared to disposing of it externally, was associated with greater human AMR carriage (OR=1.29, p=0.03,

95% CI [1.02-1.63], GLMM) (Table 4.4). My results were consistent when separate analyses for the individual resistances was performed (Table B3 in Appendix B).

Table 4.4. Results of two generalized Poisson Mixed Models investigating household risk factors for AMR carriage in humans at the household level. Households not keeping livestock used as the reference level in Model 1.

Model 1: AMR carriage, humans in all households	**Estimate**	**Standard error**	**P value**
Human density	0.23	0.08	0.003
Large livestock (with or without small livestock)	-0.14	0.12	0.24
Small livestock only	0.0075	0.11	0.94
Model 2: AMR carriage, humans in livestock keeping household only			
Human density	0.24	0.09	0.009
Manure in household	0.26	0.12	0.03

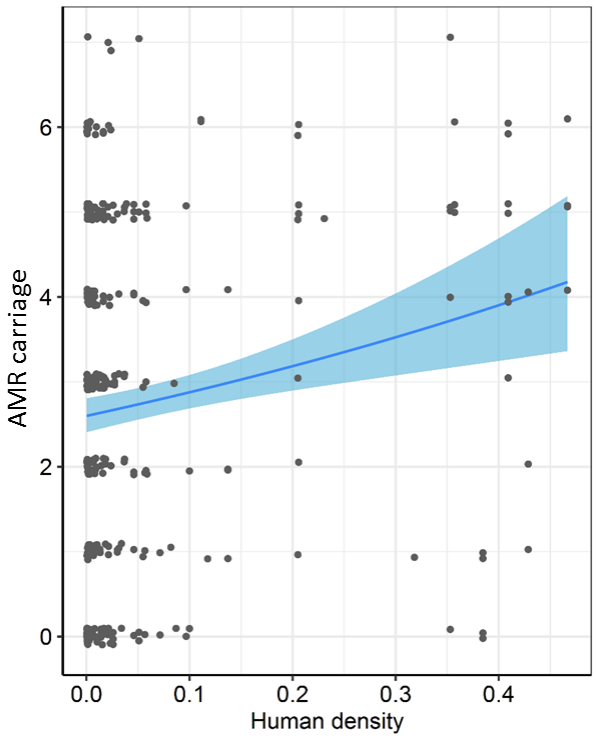

Figure 4.4 Fit of a Poisson generalised linear mixed effects model showing how increasing human density in a household influences AMR carriage in humans. All other covariates in the models are kept constant. Shading on either side of each line represents 95% confidence intervals. Points have been jittered for clarity.

4.5 Discussion

In this study I applied ecological and epidemiological approaches to characterise the epidemiology of AMR *E. coli* isolates systematically collected from sympatric human and livestock populations in the rapidly developing urban landscape of Nairobi, Kenya.

Resistance to aminoglycosides, sulfonamides, tetracyclines, trimethoprim and penicillins was high in both humans and livestock, while resistance to cephalosporins and fluoroquinolones was low. These results are consistent with previous studies (Kariuki et al., 2003; Bii et al., 2005; Adelaide et al., 2008; Oundo et al., 2008) and may in part be a reflection of the patterns of antimicrobial use in human and animal health. However, background data on antimicrobial use in these populations is limited. My results indicating a high prevalence of AMR carriage are based on non-clinical isolates from humans and livestock.

When analysed by host, human isolates appeared to have a higher prevalence of AMR carriage when compared to livestock isolates, with the exception of tetracyclines. In particular, the observed prevalence was significantly higher in four clinically relevant antimicrobial classes (penicillins, sulfonamides and trimethoprim and aminoglycosides). A possible explanation for this variation in AMR carriage is that it relates to variation in antimicrobial use between these populations. Although antimicrobials are used extensively in both human and livestock populations, previous studies have shown that frequency of use of antimicrobials is higher in human medicine than in livestock, especially in resource-poor settings (Kariuki and Dougan, 2014). Similarly, in community settings where over-the-counter access to drugs is common, it is likely that humans have access to a broader range of antimicrobials, either through self-medication or inappropriate prescribing; common practices in many low and middle-income countries (LMICs) (Omulo et al., 2015; Omulo et al., 2017). Likewise, in such settings, infections are commonly treated empirically (often

using antimicrobials) with limited microbiological investigations to ascertain the causal organism(s).

Although chloramphenicol use in food animals has been banned in Kenya (Group, 2011), I noted 3% resistance to this antimicrobial in livestock. This may be explained by the use of florfenicol, a fluorinated derivative of chloramphenicol, which shows some cross-resistance with chloramphenicol (White et al., 2000). Similarly, the observed proportions of resistance against ciprofloxacin (a quinolone antimicrobial not licensed for veterinary use) among livestock isolates is probably explained by cross-resistance with other quinolones used in veterinary medicine, such as enrofloxacin and norfloxacin.

At the household level, there is evidence of an intricate interplay between humans and livestock in relation to the development and transmission of AMR. This analysis revealed that human AMR carriage increased with number of occupants in a household, and that keeping manure inside the household compound was also significantly associated with AMR carriage in humans. In urban Nairobi, people live in a continuum of urban spaces with varying human and animal population densities, with the majority (>60%) of people living in slums (Joshi et al., 2011; Bird et al., 2017), environments characterised by small household areas and high population densities. Population density is an important factor in the population prevalence of AMR in populations (Bruinsma et al., 2003), and may in part be due to the significant correlation between overcrowding and high infectious diseases burden more broadly(Yang et al., 2012); an important driver of antimicrobial use in resource poor settings such as Nairobi. Similarly, high human populations within a household result in a greater epidemiological connectivity; thus facilitating exchange of AMR bacteria and their AMR determinants. The number of urban dwellers in the majority of LMIC cities, including Nairobi, is projected to grow significantly in the near future (UNPD, 2014a). While this urban demographic change is unfolding, disease burden is expected to burgeon, precipitating high antimicrobial use. For

this reason, measures to curb infectious diseases burden by the public health policy makers, in part to reduce drug pressure on micro-organisms, are needed.

This results suggest that at the household level, livestock ownership in and of itself does not add to the risk of acquisition or carriage of AMR bacteria in humans. However, given the multiple pathways of AMR exchange between humans and livestock (Woolhouse et al., 2015), via the food chain or due to environmental pollution, it is possible that the direct effect of livestock keeping on proportions of AMR in humans could be confounded by other factors not captured in this study. This study does, however, suggest that, whilst AMR carriage was not directly associated with the presence of livestock in the household, the impact of keeping livestock on human AMR carriage was mediated by some practices associated with livestock keeping, namely the presence or absence of animal manure in the household. These results support other studies that have identified animal manure as a reservoir of AMR bacteria and AMR determinants (Udikovic-Kolic et al., 2014; Graham et al., 2016). Importantly, amplification and persistence of AMR determinants such as AMR plasmids can take place in manure and be further disseminated to humans via cross-contamination pathways such as through exposed water and food (Pornsukarom and Thakur, 2017), or via peri-domestic wildlife. Although there is still a lack of knowledge concerning the exact mechanism, particularly the genetic basis of transmission (Heuer et al., 2011), strategies that limit AMR gene flow to and from manure (to humans) should be adopted. Such measures include safe disposal of manure from households, and manure pre-treatment prior to application onto crop farms where possible.

It is important to note that, while this analysis was not designed to address transmission of AMR bacteria and their AMR determinants, it is also plausible that clonal expansion could have played a role in the observed AMR patterns. The finding of 35 common AMR profiles in both human and livestock bacterial populations may, in part, reflect overlapping antimicrobial usage patterns,

acquisition of AMR from a shared source or clonal expansion. I hypothesise that the finding that 30.5% (340/954) of all isolates contain a tetracycline-sulfonamide-trimethoprim cluster phenotype and that the pairwise correlations between these three antimicrobial classes were very high is suggestive of a conjugative MDR plasmid circulating within the *E. coli* population in both human and livestock populations. AMR genes conferring resistance to tetracycline, sulfonamide and trimethoprim antimicrobial classes are commonly associated with mobile genetic elements (Harmer and Hall, 2015), and these elements play a pivotal role in dissemination of MDR in *E. coli* isolates. Genetic data is required to validate the existence of mobile genetic elements, and determine whether AMR genes are being transferred across them.

Distinguishing molecular transmission of AMR, from selection for AMR due to antimicrobial use, is challenging (Muloi et al., 2018). In particular, in an urban environment such as Nairobi, where human habitation, livestock keeping, and food supply chains are interconnected (Alarcon et al., 2017) the relative contributions of the aforementioned drivers are difficult to quantify. At a finer scale, any study investigating the transmission of AMR between humans and livestock in these low resource settings needs to consider indirect transmission, rather than just direct animal to human and/or human to animal transmission. Whilst direct host-to-host transmission of AMR bacteria and AMR determinants may occur, in these intricate ecosystems, the role played by the wider environment (e.g. wildlife, soil and, in particular, hospital and farm effluents) in relation to acquisition of AMR from a common source may be vital.

4.6 Conclusion

Taken together, using a rigorously-structured epidemiological study design, I report a high prevalence of AMR *E. coli* carriage in livestock and humans outside the clinical setting across a developing-country urban landscape, with no evidence that direct contact with livestock contributes to the burden of human AMR, but that indirect contact between livestock and humans does play a role.

In LMIC urban ecosystems, the elevated prevalence of AMR in both human and livestock populations could be attributed to unregulated access to antimicrobials, poor hygiene and sanitation, and waste management, which encourage the evolution and spread of AMR bacteria. These findings highlight a need for targeted surveillance strategies across various sectors, and for actors to address and design effective measures to curb AMR in these populations, both in Nairobi and in other similar urban landscapes. Further work is required to understand the ecology of genetic determinants of resistance, in particular the extent of the role plasmids play in the dissemination and evolution of resistance traits in these human and livestock populations.

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Chapter 5

One Health genomic epidemiology of antimicrobial resistant *Escherichia coli* carriage in co-habiting human and livestock populations

> People and gorillas, horses and duikers and pigs, monkeys and chimps and bats and viruses: We're all in this together.
>
> **David Quammen, 2012**

Chapter 5 One Health genomic epidemiology of antimicrobial resistant *Escherichia coli* carriage in co-habiting human and livestock populations

5.1 Abstract

Livestock have been proposed as a reservoir for AMR bacteria and AMR genetic determinants that may infect humans, yet quantitative evidence regarding their epidemiological role remains lacking. I used a combination of genomics, epidemiology and ecology to investigate patterns of AMR carriage in *E. coli*, regarded as a sentinel organism. I conducted a structured epidemiological survey of 99 households across Nairobi, Kenya and whole genome sequenced one *E. coli* isolate from 315 human and 594 livestock faecal samples.

I detected high rates of AMR gene carriage, 60 different acquired genes and 14 point mutations, and found that 10/74 of the genes were significantly more common in human than in livestock isolates. Highest AMR gene carriage was observed in humans, pigs and poultry compared to rabbits, goats and bovines. AMR gene community composition was not associated with host type or household location. I found that, whilst AMR gene carriage in humans was not directly associated with the presence of livestock in the household, the impact of keeping livestock on human AMR gene carriage was instead influenced by livestock-keeping practices, in particular the presence or absence of animal manure in the household.

In conclusion, I did not find any evidence to support the hypothesis that the keeping of livestock is a risk factor for emergence and dissemination of AMR genes to humans in this setting. The characterisation of AMR patterns in which co-habiting human and livestock populations were systematically sampled provides new insight into the broader epidemiology of AMR in complex and interconnected urban environments.

5.2 Introduction

Humans and livestock are linked in many ways including direct contact via close proximity, and shared environments receiving human and animal waste. The close contact that people have with livestock could provide an opportunity for either population to act as a reservoir from which AMR bacteria or their AMR determinants could be transmitted in either direction, (Planta, 2007; Hassell et al., 2017). The tangled interplay of AMR transmission between people and livestock is likely to be most acute in LMICs making investigations of AMR transfer in these settings a public health priority. Livestock have been proposed as a source for AMR bacteria in humans, with the keeping of livestock widely believed to be a risk factor for AMR in humans (Bélanger et al., 2011; O'Neill, 2015). However, quantitative evidence describing the role of livestock in the emergence and transmission of AMR bacteria and their resistance determinants to human populations is lacking (Muloi et al., 2018; Hanage, 2019).

Current surveillance of AMR *E. coli* tends to focus on tracking specific AMR genes (mostly of clinical importance such as ESBLs) in either human or livestock populations without making comparisons of resistances between the two populations (Muloi et al., 2018). Whole genome sequencing of spatiotemporally-related isolates from human and livestock populations overcomes both of these limitations and may help us to improve our understanding of how and where AMR develops and spreads across different host populations or niches.

In this study, using whole genome sequence analysis of *E. coli* isolates obtained from cohabiting human and livestock populations; I determined the prevalence and mechanisms of resistance, and characterised AMR gene diversity and structure of AMR genes in the different host populations. At a finer scale, epidemiological models were used to investigate risk factors for exchange of AMR genes between sympatric humans and livestock, thus shedding light on pathways of AMR transfer at household interfaces.

5.3 Material and methods

Details on study design, sample collection, bacterial isolation, whole genome sequencing and bioinformatic analysis are presented in chapter 2.

5.3.1 Detection of antimicrobial resistance genes

Acquired genes encoding antimicrobial resistance were identified through the ResFinder tool (Zankari et al., 2012a) via the Center for Genomic Epidemiology batch upload platform (https://cge.cbs.dtu.dk/services/cge/). The threshold of AMR gene detection was set to 90% identity and 60% coverage, as this is shown to be the optimal threshold for this method (Zankari et al., 2012b). The 60% coverage threshold was to ensure that AMR genes spread over two contigs, and/or located on the edge of the contig were not missed. As resistance to some of the antimicrobial agents, in particular fluoroquinolones, can be caused by point mutations (Single Nucleotide Polymorphisms (SNPs)), PointFinder (Zankari et al., 2017) was used to detect point mutations known to confer antimicrobial resistance.

5.3.2 Distribution of AMR genes by host types

Differences in the distribution of AMR genes between hosts were calculated using Chi-squared tests (humans and livestock), and one way ANOVA (human vs different livestock groups) using R package stats (R Core Team, 2013). Tukey's multiple-comparison test was performed *post-hoc* for pairwise comparisons between groups, and P values of <0.05 were considered significant.

5.3.3 Alpha diversity

Comparisons of alpha diversity between host groups were conducted using richness (defined as the number of unique AMR genes in an isolate), Simpson diversity index (1-D), and Shannon's index using the diversity function in the vegan package (Oksanen et al., 2015). Mann–Whitney U test was used for comparisons between human and livestock. For comparisons of more than two

groups, Kruskal–Wallis test was applied, and statistical differences were corrected for a multiple comparison test using the Bonferroni correction.

5.3.4 Rarefaction analysis

To estimate whether or not differential sampling bias could be, in part, responsible for the observed diversity of resistance determinants in the isolates I performed a rarefaction analysis (Hughes et al., 2001) using the R package iNEXT. Sample-based curves evaluated the number of AMR genes in a sample by plotting diversity estimates in relation to the number of sampling units. All extrapolation curves were plotted using a doubling in sample size, and 999 bootstrap replicates were used to estimate 95% confidence intervals. Ninety-five percent confidence intervals were used to determine if differences between methods were statistically significant.

5.3.5 Beta diversity

To complement alpha-diversity analyses, I evaluated whether AMR gene communities in human and livestock (and the different livestock groups) populations had different structures (beta-diversity). AMR gene counts for each antimicrobial class were computed and Hellinger transformed to avoid overweighting of rare AMR genes. Bray-Curtis abundance-based dissimilarity matrix (by antimicrobial classes) was highly correlated with Jaccard incidence-based matrix (AMR gene presence and absence) ($r = 0.81$, $P < 0.001$; Mantel test), so I used a Bray-Curtis abundance-based dissimilarity matrix.

Bray-Curtis dissimilarity between all samples was calculated using the R package vegan. Average-linkage clustering was used for all hierarchical sample clustering. A circular Bray-Curtis AMR gene community dendrogram was constructed by exporting the dendrogram in Newick format using the ape package (Paradis et al., 2004) and displayed using Interactive Tree of Life tool (Letunic and Bork, 2016).

Further, Non-Metric Multidimensional Scaling (NMDS) was used to ordinate and visualize the Bray-Curtis distance matrix. Confidence ellipses were drawn

around samples from each host group, using a 95% confidence interval. Ordination was performed separately for humans compared to livestock, and humans compared to the different livestock groups.

5.3.6 Genetic differentiation and structure of AMR genes by household location

Here I investigated genetic differentiation and structure of AMR genes within and between host groups from the same household and those from different households. I hypothesise that within the same household (across different hosts) AMR gene dispersal is widespread and driven by continuous immigration of mobile genetic elements and AMR bacteria. Specifically I ask, what is the level of AMR gene variation within and between hosts in the same household and those from different households? I.e. are AMR gene communities from one household genetically more similar than two individuals chosen from two different populations? To empirically and quantitatively test the relative contribution of geographical location (i.e. within the same household and different households) in structuring AMR gene assemblage, intra and inter-household Bray-Curtis dissimilarities were compared using permutation tests. Permutation tests were calculated for, i) human samples only, ii) livestock samples only, iii) human and livestock samples, and iv) human and the various livestock groups i.e. human and poultry. The statistical significance was tested using 10^6 permutations.

5.3.7 Modelling AMR gene sharing between humans and livestock at the household level

To investigate the potential drivers of AMR gene carriage in humans at the household level I used a zero inflated General Linear Mixed Model (GLMM) with counts of the individual AMR genes (also referred to as AMR gene length) in each of the isolated aggregated at the antimicrobial class level as the dependent variable. Risk factors analysed included: human density (persons in a household as a function of household area) and kinds of livestock kept in the household

(small livestock only, large livestock +/- small livestock, and no livestock). Moreover, for households that kept livestock, a separate zero inflated poison GLMM was fitted to investigate the influence of human density and manure disposal practises (manure disposed in the household compound or outside) on AMR gene length. Separate models were fitted for the most prevalent AMR genes.

I first looked for collinearity among our covariates using bivariate correlations. Covariates had correlation coefficients 0.3 or less indicating collinearity was not a serious concern. Analysis were performed using the glmmTMB package (Magnusson et al., 2017) in R. I plotted the diagnostic plots of the zero inflated Poisson mixed model, including random effects, to check that the model assumptions were not violated using the R package sjPlot (Lüdecke, 2017). To account for the nested (or hierarchical) nature of our sampling design household site (n=99), sublocation (n=33) and wealth category (n=7) were included as random effects. To estimate the variance due to differences in carriage between antimicrobial classes, antimicrobial class was included as a random effect. To account for variation due to genome assembly and AMR gene prediction all models were offset by number of contigs.

5.4 Results

5.4.1 Antimicrobial resistance genes characterisation

A total of 909 isolates composed of 315 human and 594 livestock isolates (Table 5.1) were whole genome sequenced and screened for known genetic determinants of AMR, including acquired AMR genes and point mutations in chromosomal genes associated with AMR.

Table 5.1 Number of human and livestock isolates collected from the 99 households from Nairobi, Kenya (2015-2016). Livestock isolates are broken down by source.

Source		Number of isolates	% of isolates
Human		315	34.7
Livestock:		594	65.3
	Poultry	314	34.5
	Bovine	61	6.7
	Goat	128	14.1
	Pig	50	5.5
	Rabbit	41	4.5

I detected 60 acquired genes and 14 point mutations (7 in *parC* region, four *gyrA*, two *parE*, and one *ampC*) known to confer resistance to 9 antimicrobial classes. Across all isolates, the most common AMR genes were, *sul2* (46%), *strA* (41.1%), *strB* (41.1%), *tetA* (38.3%), and $bla_{TEM\text{-}1B}$ (25%) conferring resistance to sulphonamides, aminoglycosides, tetracyclines and β-lactams respectively (Figure 5.1). Three hundred and one (41.4%) *E. coli* isolates analysed were pan-susceptible. A significantly larger proportion of livestock isolates (45.7%) compared to human isolates 102 (31.9%) did not contain a single AMR gene (or point mutation) ($p<0.001$, Fisher's Exact test). Of the 289 pan-susceptible livestock isolates, 31.6% of poultry isolates (n=106), 65.1% of bovine isolates (n=41), 34% of pig isolates (n=17), 70.6% of goat isolates (n=96), and 70.7% of rabbit isolates (n=29) were pan-susceptible.

For the majority of the predicted genes the distribution did not significantly differ between human and livestock isolates. However, the abundance of 10/74

genes (*sul1, sul2, strA, strB, bla*$_{TEM-1B}$, *mphA, dfrA7, dfrA8, gyrA_S83L*, and *catA1* genes was higher in human isolates as compared to livestock isolates (p<0.05, Bonferroni correction).

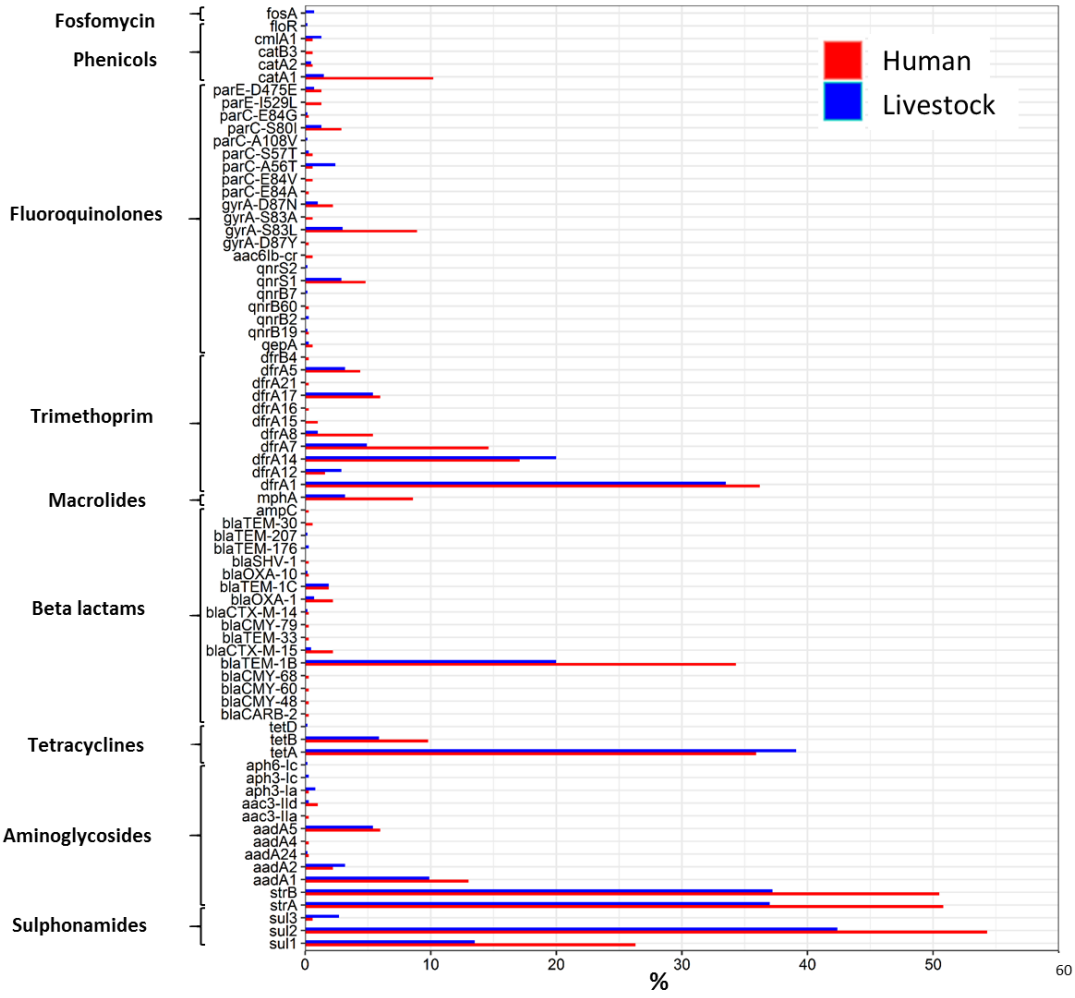

Figure 5.1 Prevalence of 74 AMR genes and point mutations associated with reduced antimicrobial susceptibility in 315 human and 594 livestock *E. coli* isolates collected from the 99 households from Nairobi, Kenya (2015-2016).

Considering the heterogeneous nature of "livestock" as a group, I further investigated differences in the distribution of AMR genes between human and the different livestock groups. Significant differences in the proportion of AMR genes were noted in 10 of the 60 acquired genes. These included *tetA, sul1, sul2, strA, strB,* $bla_{TEM\text{-}1B}$*, dfraA1, dfrA7, dfrA14* and *catA1*. Tukey's *post hoc* test revealed significant differences in the proportion of resistance between different pairs of host groups for the ten AMR genes (Figure 5.2, Table C1 in Appendix C). Overall, for all AMR genes expect *dfrA14*, humans had a significantly higher proportion when compared to bovines, pigs or rabbits. Conversely, pigs and poultry had a significantly higher proportion of *strA*, and *tetA* and *dfrA14* genes respectively when compared to humans.

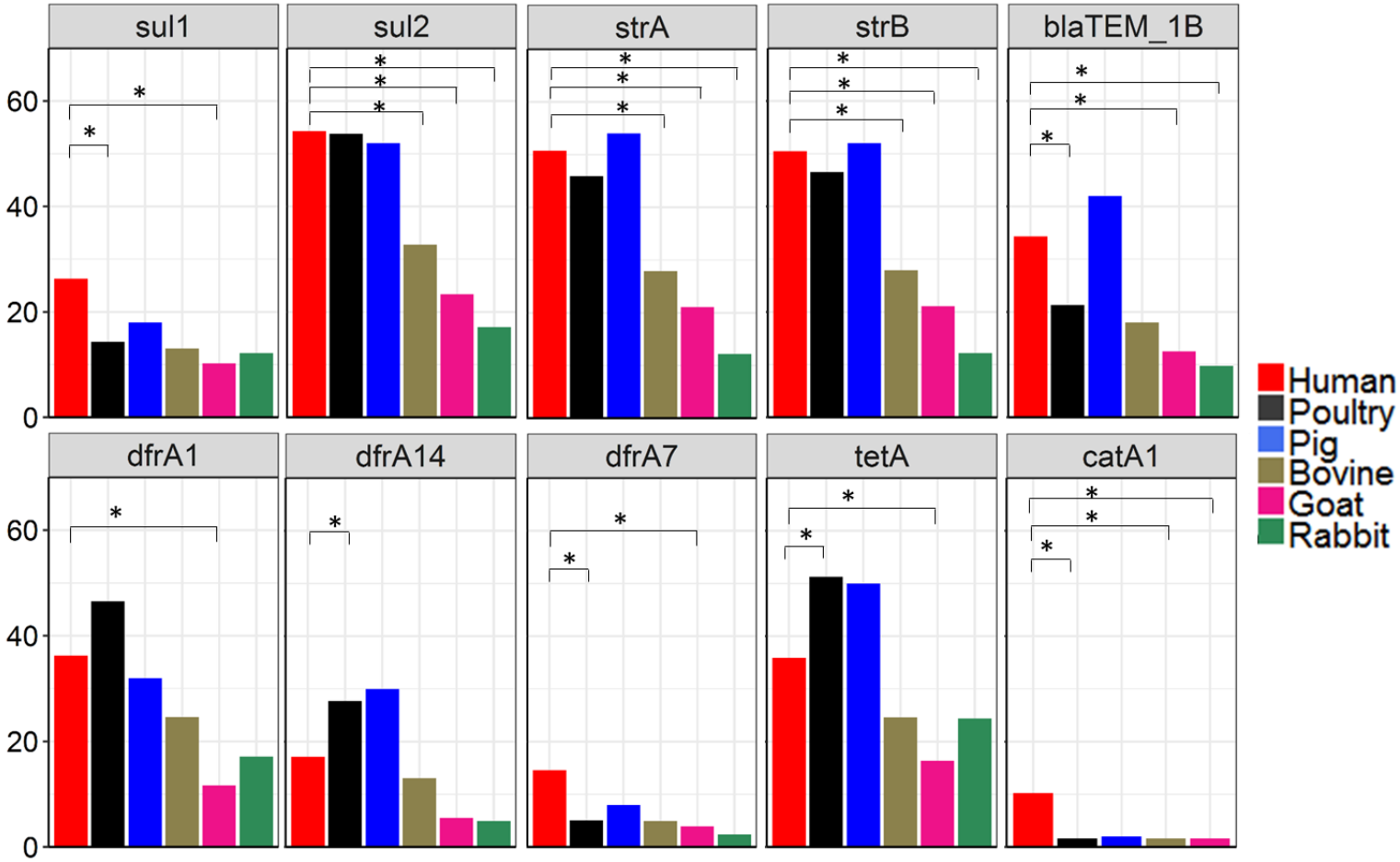

Figure 5.2 A subset of genes that differed significantly in abundance between at least two host groups. * P < 0.05, Kruskal test, Bonferroni correction

5.4.2 AMR gene abundance and diversity

Overall, AMR genes were more common in human when compared to livestock isolates (median 5 vs. 1; p<0.001, Man-Whitney U test) (Figure 5.3). When compared to the different livestock groups, human carriage of AMR genes was not significantly different from poultry and pigs (p>0.05, Kruskal Wallis, Bonferroni correction). However, human isolates had higher carriage when compared to bovine, goat and rabbit isolates (p < 0.05, Kruskal Wallis, Bonferroni correction).

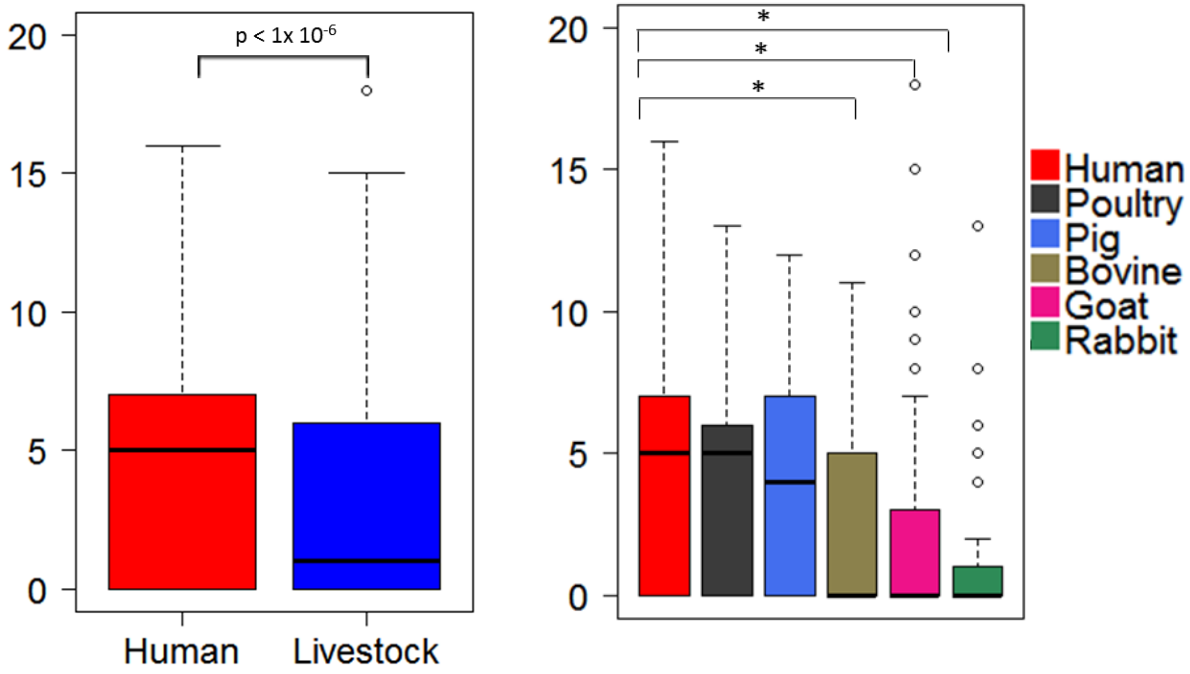

Figure 5.3 Number of AMR genes per isolate in a) humans and livestock, b) humans and the different livestock groups P values were calculated using the Wilcoxon test and Kruskal Wallis. Numbers indicate AMR gene numbers within each group.

This pattern was consistent with Shannon and Simpson, diversity indices, which assess both AMR gene abundance and richness (Figure 5.4).

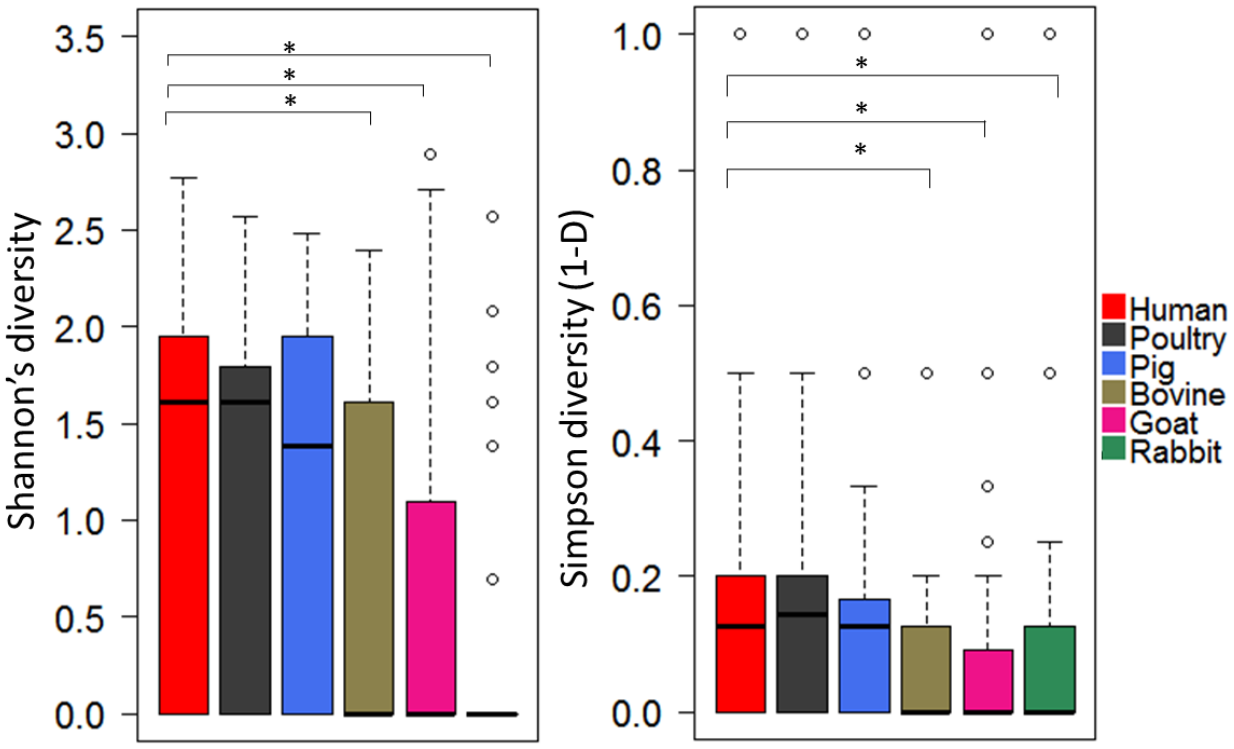

Figure 5.4 Box plot of A) Shannon's' diversity, and B) Simpson diversity (1-D) at the AMR gene level of the various hots groups. The horizontal line is the median value, the middle box indicates the inter-quantile range (IQR), whiskers represent values within 1.5 IQR of the lower and upper quartiles, and individual points show outlier values.

5.4.3 Rarefaction analysis to evaluate effect of sampling effort on diversity

Rarefaction analyses were used to evaluate if the sampling effort was sufficient to fully capture the diversity of AMR genes in both human and livestock *E. coli*. Overall, it showed that AMR gene diversities were slightly higher in human isolates compared to livestock, when sampled at comparable depths. However, despite the depth of our sampling, diversities estimates still represent the lower bounds of the "true AMR gene diversity". Asymptotic estimates of AMR diversity, according to the rarefaction analyses, for human and livestock was approximately 4000 and 5000 samples respectively, indicating that more sampling is needed (Figure 5.5).

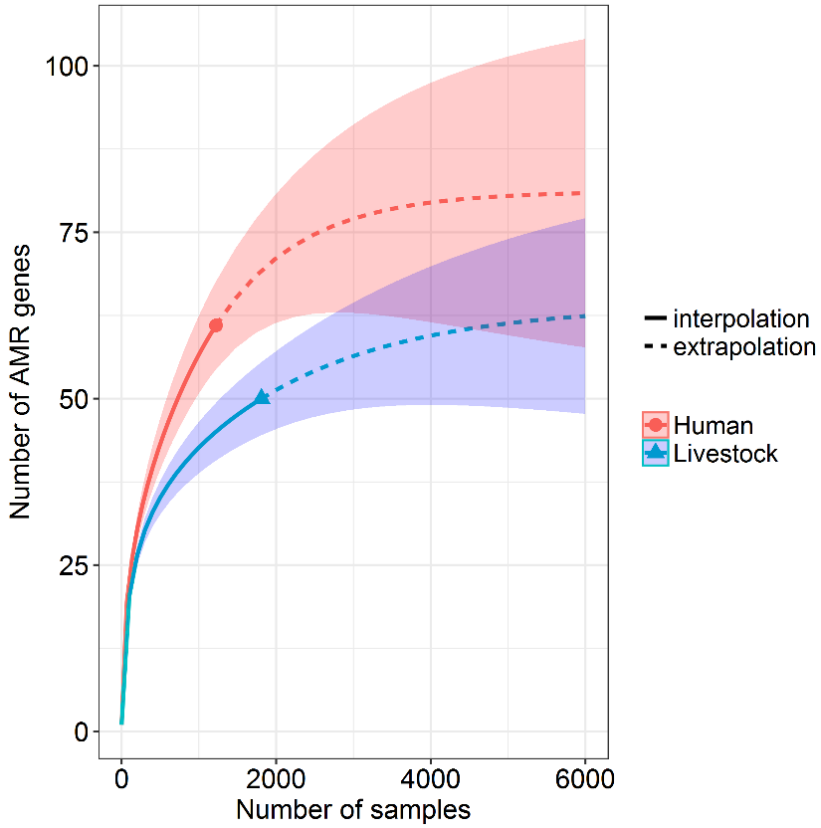

Figure 5.5 Sample-based rarefaction curve to assess saturation and estimate the total richness (number of AMR genes) predicted in our dataset. Solid black line is the rarefaction curve. Dotted line indicates Chao2 estimation of asymptotic richness by number of samples, and shading indicates 95% confidence intervals.

5.4.4 AMR gene community assemblage

First, I calculated the Bray Curtis dissimilarities between the gene-level AMR gene compositions for all isolates and visualised it in a dendrogram. There was no obvious separation by host species (Figure 5.6).

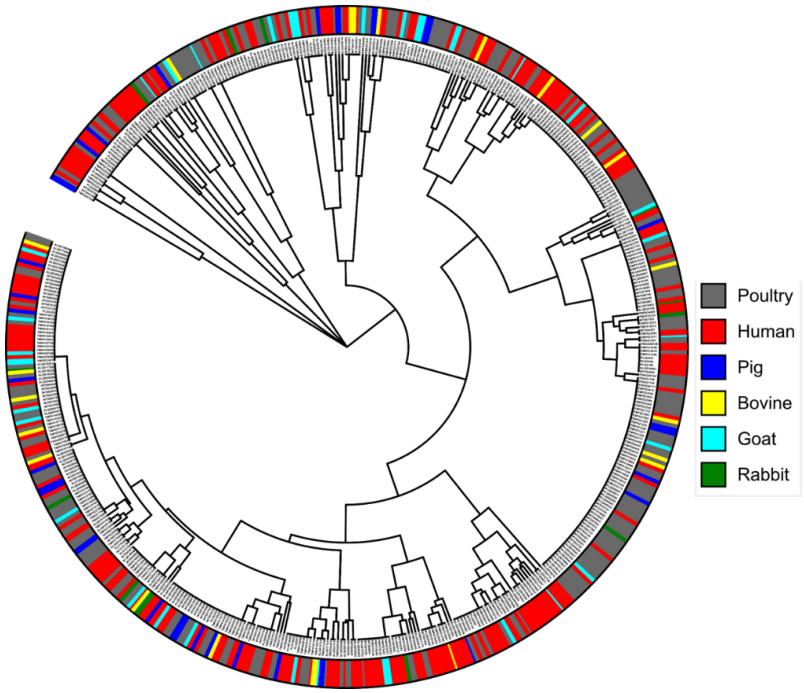

Figure 5.6 A dendrogram showing the AMR gene assemblage for human, poultry, pig, bovine, goat, and rabbit AMR gene communities using average linkage of Bray Curtis dissimilarities.

Next, I assessed differences in overall AMR gene community composition in human and livestock (and the different livestock groups). I used techniques that are based on the relative abundance of AMR genes within communities and the extent of genetic divergence between AMR genes. The NMDS ordination in two dimensions adequately represented data on the AMR gene community structure, as evidenced by low stress values (0.08). The 95% confidence ellipses for the centroids of human and livestock overlapped indicating that AMR gene assemblages in both human and livestock were similar to each other (Figure 5.7a). Similarly, when compared to the different livestock groups, ordination analysis showed that AMR gene community assemblage was not significantly different between humans and the different host groups (Figure 5.7b).

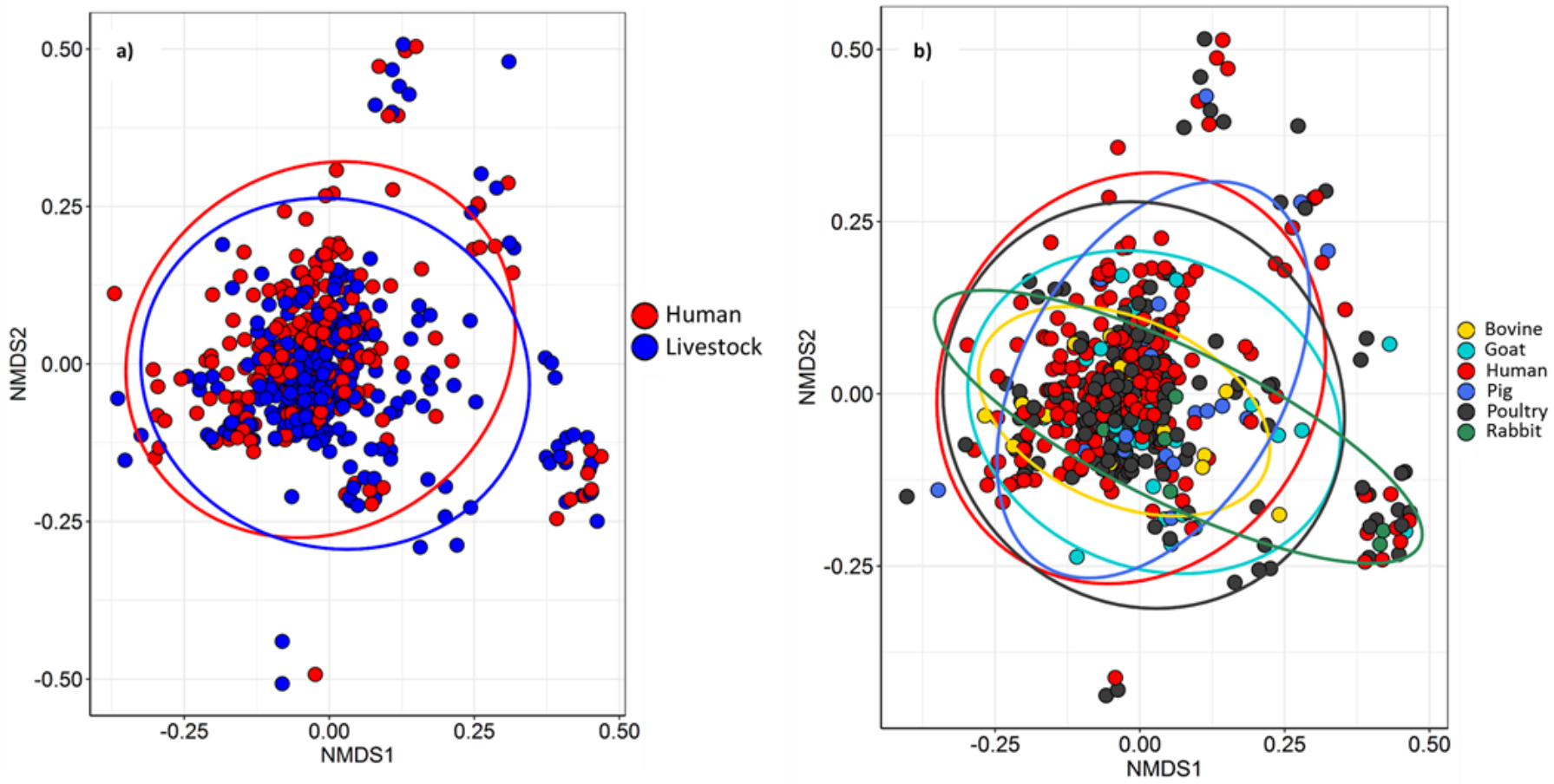

Figure 5.7 Non-metric multi-dimensional scaling (NMDS) of a Bray-Curtis distance matrix describing AMR gene communities in a) human and livestock isolates, and b) human and different livestock groups. Each point symbolizes an isolate's AMR community, some points may overlap completely. Ellipses represent 95% confidence estimates for mean NMDS scores. Non-overlapping centroids are considered significantly different at a = 0.05

5.4.5 AMR gene assemblage at the household level

Multivariate permutation tests indicated that AMR gene community in human and livestock isolates from the same household and those from different households didn't not differ ($p > 0.5$, permutation test) (Figure 5.8a). Similar pattern was observed when analysing human and the specific livestock groups. Livestock isolates from the same households tended to have similar AMR gene composition ($p<0.01$); however this was not evident in human only isolates ($p >0.05$) (Figure 5.8b-c).

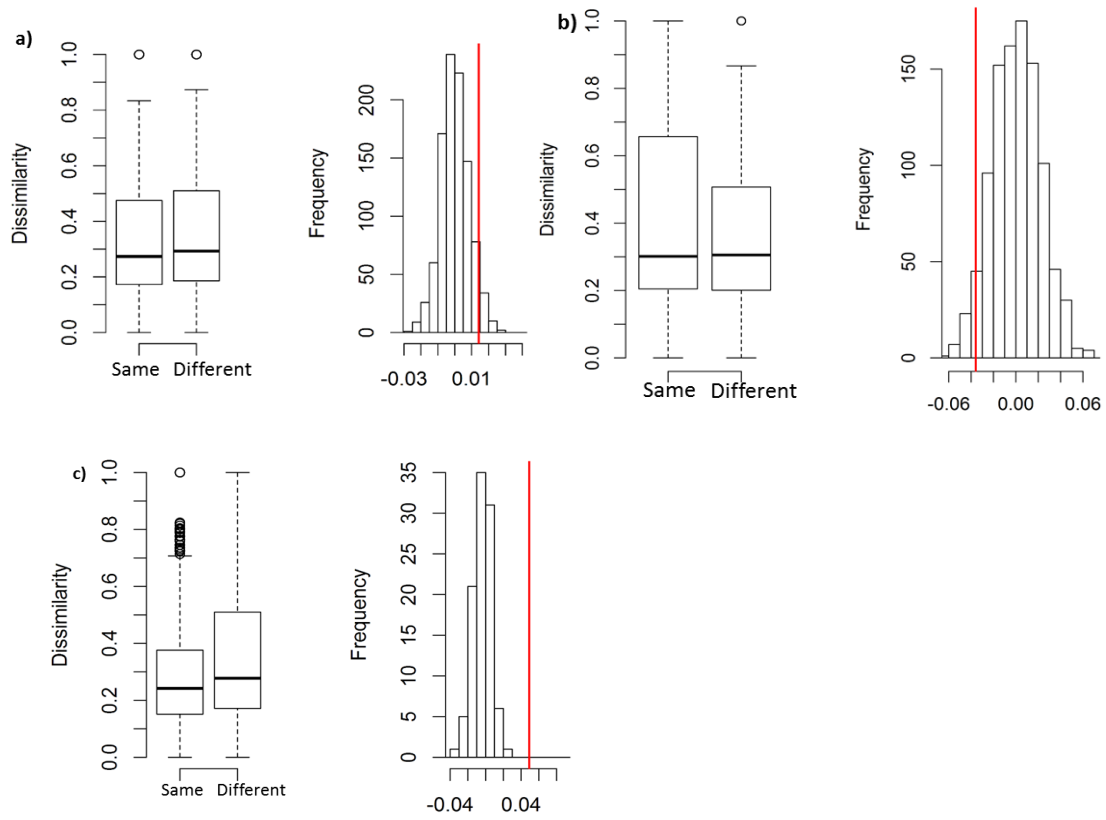

Figure 5.8 Comparison of the observed values (red line) with the null distribution of the differences in dissimilarity in AMR gene communities within and among households for a) both human and livestock, b) human only, c) livestock only isolates.

5.4.6 Role of livestock keeping in AMR gene carriage in humans at the household level

Having established that AMR gene communities were similar in humans and livestock, I next aimed to assess whether livestock keeping, and livestock keeping practices, influenced AMR gene carriage in humans. The AMR gene assemblage in humans was analysed with respect to livestock keeping status using nonmetric multidimensional scaling (nMDS) and analysis of similarity. The 95% confidence ellipse for the centroid of humans keeping livestock overlapped with that of humans not keeping livestock (Figure 5.9). Likewise, Permutational Multivariate Analysis of Variance (PERMANOVA) showed that the AMR gene composition of humans keeping livestock did not differ from that of humans not keeping livestock (adonis R^2= 0.0097, p =1).

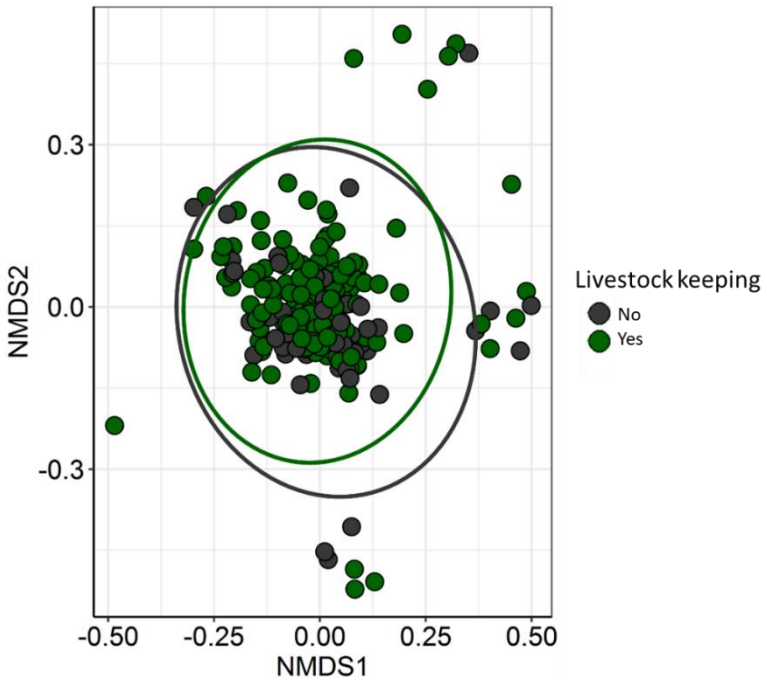

Figure 5.9 Non-metric multi-dimensional scaling (NMDS) of a Bray-Curtis distance matrix describing AMR gene assemblage in humans with respect to the livestock keeping status. Each point symbolizes an isolate's AMR community, some points may overlap completely. Ellipses represent 95% confidence estimates for mean NMDS scores.

Considering the variation in the different livestock groups kept across the households, I analysed the AMR gene assemblage in humans with respect to the kind of livestock kept in the household (poultry, bovines, pigs, rabbits and goats). Consistently, the AMR gene composition of humans did not vary depending on the kind of livestock kept in the household (Adonis R2 <0.01, p= 1).

In agreement with the NMDS and PERMANOVA analysis, using zero inflated generalised linear mixed models, the results suggest there was little evidence (if any) that the presence of livestock (small or large+/-small) influences the risk of human AMR gene carriage (p>0.05) (Model1,Table 5.2; Figure 5.10a). The impact of livestock was influenced by the use of manure: the risk was significantly higher if manure was kept inside the household perimeter compared to disposing of externally (OR=2.13, p=0.008, 95% CI [1.22-3.71]) (Model 2, Table 5.2; Figure 5.10b), however this was rarely significant when I performed separate analyses for the individual AMR genes (Table C1 in Appendix C).

Table 5.2. Results of a zero inflated generalised linear mixed models investigating household risk factors for AMR gene carriage in humans at the household level. Households not keeping livestock used as the reference level in Model 1.

Model 1: AMR gene length, humans in all households	**Estimate**	**Standard error**	**P value**
Human density	0.08	0.21	0.72
Large livestock (+/- small livestock)	-0.08	0.3	0.78
Small livestock only	-0.19	0.27	0.47
Model 2: AMR gene length, humans in livestock keeping households only			
Human density	0.3	0.25	0.2
Manure in household	0.75	0.28	0.007

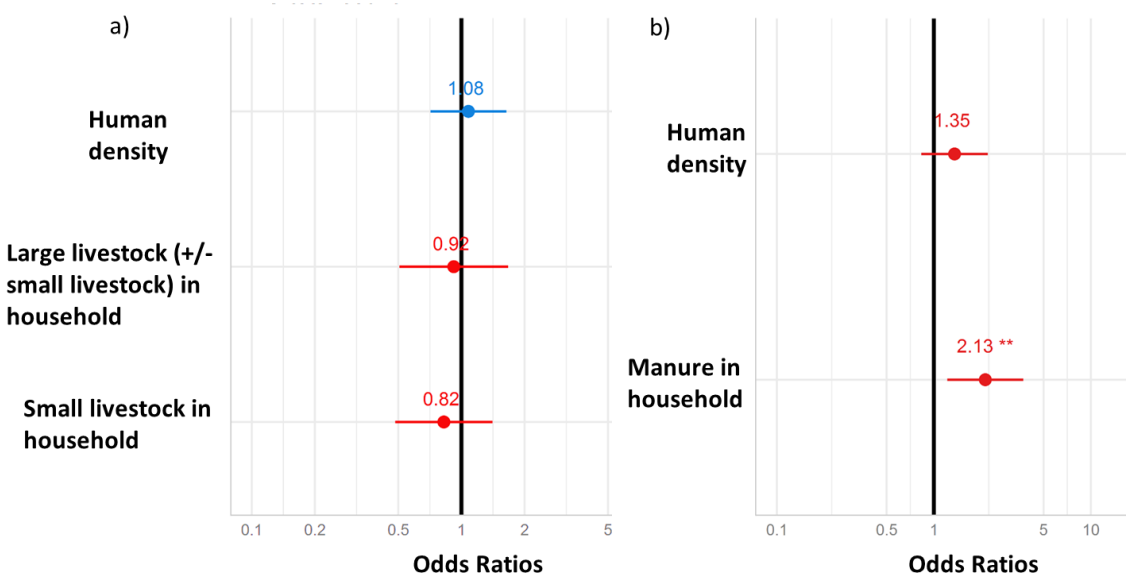

Figure 5.10 Fit of the zero inflated generalised linear mixed models. a) Model 1: AMR gene length, humans in all households, b) Model 2: AMR gene length, humans in livestock keeping households only.

5.5 Discussion

In this study I investigated the epidemiology of AMR gene carriage in *E. coli* isolates systematically collected from sympatric human and livestock populations in the rapidly developing urban landscape of Nairobi, Kenya, using a "One Health" approach.

I detected 74 AMR genes (60 acquired and 14 point mutations associated with reduced antimicrobial susceptibility) known to confer resistance to nine antimicrobial classes. The most common AMR genes were *sul2, strA, strA, tetA* and $bla_{TEM\text{-}1B}$ conferring resistance to sulphonamides, aminoglycosides, tetracyclines and β-lactams antimicrobial classes all of which are commonly used in both livestock and humans. This observed prevalence of AMR genes carriage in non-clinical human and livestock isolates highlights the need for continued monitoring of the prevalence of ARGs through the continuum of ecosystems, in line with the One Health initiative. When analysed by source, most (64/74, 86.5%) of the genes were found in similar proportions in both humans and livestock, highlighting their ubiquitous distribution in both reservoirs, while the remainder (10/74, 13.5%) were significantly more common in human than in livestock isolates. The greater abundance and diversity of AMR genes in humans, chickens and pigs than in bovines, goats and rabbits can be explained by; (i) the high antimicrobial usage in humans and in intensively farmed pigs and poultry and less so in extensively farmed cattle and goats (Kariuki and Dougan, 2014; Woolhouse et al., 2015) – although such data are lacking; and ii) frequent contact between humans, chickens and pigs than for humans and goats, rabbits and cattle.

Further evidence for AMR gene similarity between humans and livestock was provided by comparisons of human and livestock AMR gene communities, findings which demonstrated that human and livestock isolates have overlapping AMR gene communities. In complex urban interfaces such as Nairobi where

humans and livestock are linked in many ways, including direct contact, consumption of livestock products by humans, horizontal gene transfer of AMR genes and vertical AMR transmission could explain this phenomenon. Further, given the shared environments receiving human sewage and manure from livestock, it is possible that acquisition of AMR from a common AMR gene 'pool' can also play a role in the observed overlap. It might also be speculated that selection for AMR due to overlapping patterns of antimicrobial use in both human and livestock populations might explain the similarity in AMR gene communities. It was not possible to distinguish between hypotheses using the data in this study.

The results of this study also indicate that AMR gene communities in livestock hosts are structured by the household scale whereas humans shared similar AMR genes outside the household confines quite frequently. In addition to the highly complex and interconnected food supply chains, humans often move between the sublocations enabling dispersal of AMR bacteria and AMR determinants across the fragmented urban landscapes. Conversely, livestock are more stationary, moving only very rarely between households themselves hence reducing the probability of AMR gene dispersal.

I found that the AMR gene communities of humans keeping livestock were not different from those of people not keeping livestock. This contradicts previous investigations where animals that live with humans are seen as reservoirs of AMR (Jakobsen et al., 2010; Leverstein-van Hall et al., 2011; Lazarus et al., 2015) but in agreement with other studies that have found limited contribution of livestock to acquisition of AMR bacteria and AMR genes in humans (Ludden et al., 2019b; Nguyen et al., 2019). The finding that livestock keeping had no direct effect on levels or communities of AMR genes confirms the observed ubiquitous distribution of AMR genes in humans and livestock but does not provide evidence of transmission of AMR bacteria or genes between the two reservoirs. To

investigate this further, I hypothesized that sharing would be associated with transmission via the same or highly related mobile genetic elements (MGEs) (discussed in detail in chapter 8).

Conversely, my analysis suggest that AMR carriage in humans was instead influenced by livestock-keeping practices, in particular the presence animal manure in the household. The deposition of manure generated in livestock production systems into the environment is identified as a potential pathway for amplification and persistence of AMR determinants (Udikovic-Kolic et al., 2014; Graham et al., 2016). These genes often located on MGEs such as broad-host-range plasmids have the potential to survive in soil, and thus potentially transferred to humans via cross-contamination pathways such as through exposed water and food (Binh et al., 2008; Huijbers et al., 2015a). However, the relative role of manured soils in the dissemination of AMR determinants to humans remains unknown and may still be highly underestimated (Baker et al., 2016). This could have important implications for urban public health.

By only sequencing a single *E. coli* isolate from each host, the within-host genetic diversity of *E. coli* was not considered in this study. Recent studies have revealed that in some bacteria is there is considerable within-host diversity and AMR mechanism diversity (Stoesser et al., 2015). In this study, the decision to sequence a single isolates from each host was made as a cost-based trade-off between the depth of sampling *E. coli* genetic diversity within each individual and the number of unique individuals from which samples could be included. It is possible this sampling bottleneck could have led to a signal being missed, but not changes to the outcome reported in this study. Assuming that each organism within a sample has equal chances of being isolated and cultured, arguably, by-isolate prevalence of AMR genes could reflect the AMR carriage in the sample most reliably. Future research could benefit from the recent development and use of metagenomics to

characterise the abundance, diversity and structure of the acquired resistomes (Pehrsson et al., 2016; Munk et al., 2017; Munk et al., 2018).

In this study not all possible sources of AMR *E. coli* or AMR determinants for humans were included (for example, wildlife, environment and importantly food of animal origin). Additional studies are required to determine the relative contribution of these possible sources of AMR *E. coli* or AMR determinants for humans. Crucially, the role of wildlife populations which are closely associated with human and livestock populations and might act as long-distance dispersers of AMR should be investigated further. Further studies are required to understand whether our findings will be reproduced in other geographical areas, and to investigate whether the noticed AMR carriage in both populations is transient or a more permanent colonization.

Currently, AMR phenotypes are monitored routinely in most clinical settings; however, this study indicates that it also will be vital to perform active surveillance for AMR determinants in the community. Importantly, this study shows that genomic analysis of specific resistance markers can augment surveillance efforts of AMR bacteria and AMR genes circulating in different ecosystems and help to implement timely control strategies designed to mitigate risks to public health (Baker et al., 2018b).

Most low-income settings in which humans and livestock live in close proximity are likely to present heightened, but to date poorly studied, risks for the evolution and transmission of AMR. By stepping outside of the 'blame game' of livestock, and medical systems, this study applying a 'One Health' approach demonstrates that AMR genes conferring AMR to critically important antimicrobials for both human and veterinary medicine are widespread in humans and livestock. Second, whilst I identified a high prevalence of shared AMR genes between livestock and humans and that human and livestock AMR gene communities were similar, I did not detect an association between livestock keeping and AMR gene numbers

or composition in humans. Instead, the impact of keeping livestock on human AMR carriage was mediated by practices associated with livestock keeping, namely the presence or absence of animal manure in the household. Although this study demonstrated AMR gene similarity between human and livestock isolates, a combination of strong epidemiological designs, involving longitudinal studies with repeated sampling on the same individual or animal, and high-resolution phylogeographic methods are still required to understand the exact direction of transmission. Taken together, this study serves as a model for the targeted sampling to harness the power of WGS for understanding the epidemiology of AMR across developing urban landscapes, a key in developing effective strategies to reduce the development and spread of such resistance in the future.

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Chapter 6

Escherichia coli genetic diversity and sharing in co-habiting human and livestock populations in Nairobi, Kenya

> Is evolution a theory, a system, or a hypothesis? It is much more – it is a general postulate to which all theories, all hypotheses, all systems must henceforward bow and which they must satisfy in order to be thinkable and true. Evolution is a light which illuminates all facts, a trajectory which all lines of thought must follow – this is what evolution is
>
> **Teilhard de Chardin, 1955**

Chapter 6 *Escherichia coli* genetic diversity and sharing in co-habiting human and livestock populations in Nairobi, Kenya

6.1 Abstract

Livestock have been proposed as an important reservoir for bacterial infections that may spread to humans, but previous studies defining relatedness of *E. coli* isolates from livestock and humans have largely been based on typing methodology with poor discriminatory power. Here, I use high-resolution molecular epidemiology to explore the dynamics of bacterial sharing within and between co-habiting human and livestock populations sampled across 99 households in Nairobi, Kenya, in an epidemiologically structured manner. A single *E. coli* isolate from 301 humans and 568 livestock was sequenced and epidemiological data recorded.

Phylogenetic analysis demonstrated that human and livestock *E. coli* are highly genetically heterogeneous with minimal evidence of clustering by host group. Genetic comparison revealed 91 sharing events differing by less than ten base pairs, mostly confined within households with instances of spread between households. Of these, 59 involved livestock isolates only (mostly in poultry), 23 human isolates only, and 9 between humans and livestock.

The genetic analysis reveals evidence of bacterial sharing in human and livestock populations, including identification of putative human/livestock transmission. Using a 'One Health' approach this study provides an integrated evaluation of the molecular relatedness of bacteria from co-habiting human and livestock populations in a low resource urban settings. These results have significance for understanding the spread of bacteria (including AMR bacteria) as well as implications for the design of surveillance studies to capture their emergence. Future research should analyse the direction of bacterial transmission between human and livestock populations.

6.1 Introduction

Humans and livestock are linked in many ways including direct contact via close proximity, and shared environments receiving human and animal waste. This close degree of mixing and contact between livestock and humans creates epidemiological interfaces across which bacteria (including AMR bacteria) can pass in either direction (Hassell et al., 2017). However, there is little empirical evidence that directly links livestock ownership and the spread of bacteria to humans. Understanding the dynamics and processes of bacterial transmission between human and livestock populations can inform potential emergence of pathogens in urban environments.

Current understanding of transmission of *E. coli* is complicated by the fact that surveillance studies have used different molecular typing approaches (Schürch and van Schaik, 2017). Of the these methods, MLST, based on 7 housekeeping genes, is the most widely used method as it is a relatively fast, accurate, and reproducible tool, which has enabled the creation of an unambiguous nomenclature for bacterial clones. However, sequence conservation in housekeeping genes limits its discriminatory power. Recent advances in sequencing technology, such as WGS, provide an unprecedented level of resolution to infer phylogenetic relatedness, thus identifying, possible or unlikely cases of epidemiological linkages of isolates (Baker et al., 2018b). Secondly, genomic studies of bacterial spread between humans and livestock have largely focused on developed countries with industrial agricultural systems (Gouliouris et al., 2018; Ludden et al., 2019b) and have often been subject to the limitations of opportunistic sampling with little spatiotemporal overlap between humans and livestock (Muloi et al., 2018).

In this study, I utilise WGS of bacterial isolates obtained from co-habiting humans and livestock to elucidate patterns of bacterial strain sharing as a proxy for transmission potential. Specifically, the aims of the current study were to

investigate: (i) the genetic diversity of commensal *E. coli* strains, (ii) genomic relatedness of *E. coli* strains from humans and livestock, (iii) the contribution of livestock keeping to acquisition of new or more diverse *E. coli* strains. *E. coli* is found in both human and food animal populations, shares the same niche as enteric pathogens and is genetically diverse (Jaureguy et al., 2008; Tenaillon et al., 2010), hence in this study, *E. coli* was considered as an ideal organism to study the sharing of pathogens between human and livestock populations. I hypothesized that the close proximity and overlap between human and livestock populations in Nairobi, aided by the sharing of common environments, would result in similar *E. coli* profiles.

6.2 Materials and methods

Details on study design, sample collection, bacterial isolation, whole genome sequencing and bioinformatic analysis are presented in chapter 2.

6.2.1 *In silico* phylogroup and MLST analysis

In silico PCR was used to assign isolates to *E. coli* phylogroups A, B1, B2, C, D, E and F using the Clermont method (Clermont et al., 2013).

In silico MLST was performed using the batch upload mode of the Centre for Genetic Epidemiology web interface hosted by the Technical University of Denmark (https://cge.cbs.dtu.dk/services/cge/) for multi-locus sequence typing (MLST) typing. MLST results were compared to results from ARIBA (Hunt et al., 2017) using the PubMLST database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) on the short reads to help resolve the novel sequence types in the data.

Global optimal eBURST (goeBURST) algorithm (Francisco et al., 2009), as implemented in PHYLOViZ (http://goeBURST.phyloviz.net), was performed to characterise genetic relatedness of the isolates based on their allelic profiles (with respect to their number of locus variants), and to further infer simple patterns of evolutionary descent by constructing a minimum spanning tree (MST) from the eBURST data. Unknown STs were assigned unique temporary ST numbers, to

distinguish them from the STs in the database. Clonal complexes were defined as groups of closely related STs differing by no more than one allele from another member of the ST.

6.2.2 SNP analysis

Of 909 isolates, 40 were excluded from further analysis because of poor quality. For analysis of SNPs, the paired-end reads were mapped to the EC958 reference genome (GCA_000285655.3) and SNPs identified in the core gene alignment using Snippy v4.0, resulting in a final set of 184786 SNPs.

6.2.3 Maximum-likelihood phylogenetic analysis

Maximum likelihood (ML) core genome phylogenetic trees were built from the 184786-SNP alignment of all 869 isolates using FastTree v2.1.10 (Price et al., 2010). In the absence of an obvious outgroup, phylogenies were rooted at the midpoint between the two most divergent taxa in the trees. All ML trees and associated metadata, such as sequence types (STs), host type, and phylogroups, were visualized in iToLv4.3 (Letunic and Bork, 2016).

6.2.4 Identification of putative bacterial sharing

A genetic distance matrix was calculated from all pairwise allelic profile comparisons and SNPs respectively, using the library "ape" in R (Paradis et al., 2004).

Epidemiological links between each pair of *E. coli* isolate were established through a systematic comparison. Household level sharing was categorised as: within household, if a sharing event involved isolates/hosts from the same household; between household, if a sharing event involved isolates in different household. Selecting a SNP cutoff to define *E. coli* transmission clusters was informed by two criteria. First, examination of the distribution of genetic similarity between individual isolates, expressed as pairwise distances, revealed that 88% (74/84) of within-household sharing events with less than 100 pairwise SNPs differed by less of equal to 10 SNPs (Figure 6.1, Figure D1 in Appendix D). Second, given that the estimated mutation rate for *E. coli* is one SNP/core

genome/year (Stoesser et al., 2016), 10 SNPs were considered to represent possible 'recent' transmission (up to 10 years).

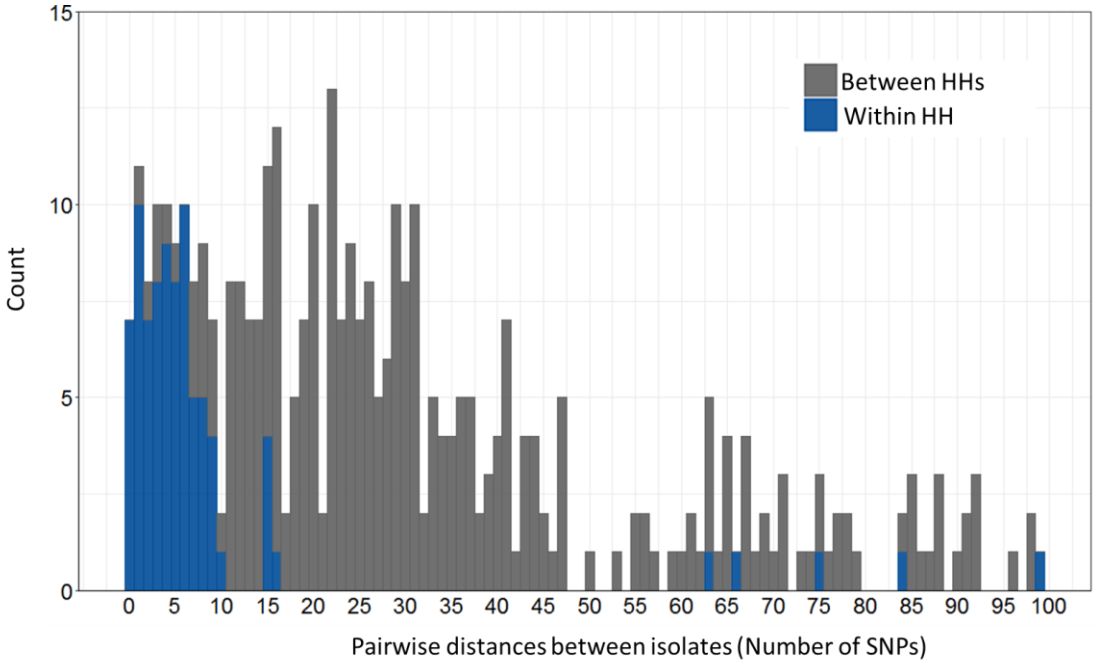

Figure 6.1 Histogram of SNP pairwise distances between isolates with differences of 0-100 core genome SNPs. Isolates from the same household indicated in blue and those of different households isolates in grey.

6.3 Results

A total of 869 genomes composed of 301 human and 568 livestock *E. coli* isolates in Nairobi, Kenya, were analysed. The number of isolates obtained from each source is presented in Table 6.1.

Table 6.1. Number of human and livestock isolates collected from the 99 households from Nairobi, Kenya (2015-2016). Livestock isolates are broken down by source.

Source	Frequency	% of isolates
Human	301	34.7
Livestock	568	65.3
Poultry	295	34
Goats	127	14.6
Cattle	59	6.8
Pigs	49	5.6
Rabbits	38	4.4

6.3.1 Phylogroup distribution

Most isolates were assigned to phylogroups B1 (n = 388, 45%) and A (n = 355, 41%). The remainder were distributed among phylogroups B2 (n = 50, 5.8%), D (29, 3.3 %), E (n = 20, 2.3%), C (n = 11, 1.3%), and F (n = 16, 1.8%). When analysed by host, the distribution of phylogroups A, C, E and F did not significantly differ between human and livestock isolates. Phylogroups B2 and D were significantly more common among human isolates versus livestock isolates and phylogroup B1 was significantly more common in livestock (Figure 6.2a) ($p<0.01$, Bonferroni correction). The identified phylogroups were present in variable proportions across the different livestock groups (Figure 6.2b).

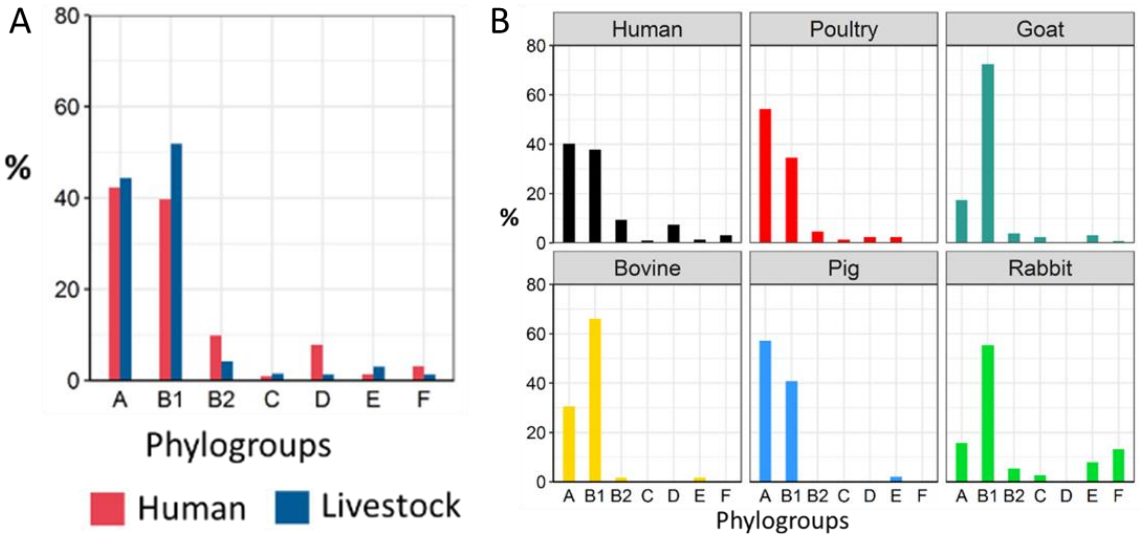

Figure 6.2 Prevalence of 7 phylogroups in a) humans and livestock, b) humans and the different livestock groups.

6.3.2 MLST distribution

The results from MLST indicated that the 301 human and 568 livestock isolates belonged to 296 different STs (Figure 6.3). No sequence type was assigned to 54 isolates that carried at least one novel allele not included in the database. Of these 54 isolates, 8.1% of poultry isolates (n=24), 6.1% of pig (n=3), 7.1% of goats (n=9), 4.6% of human (n=14), 5.1% of bovine (n=3) and 2.6% of rabbits (n=1) had no assigned STs.

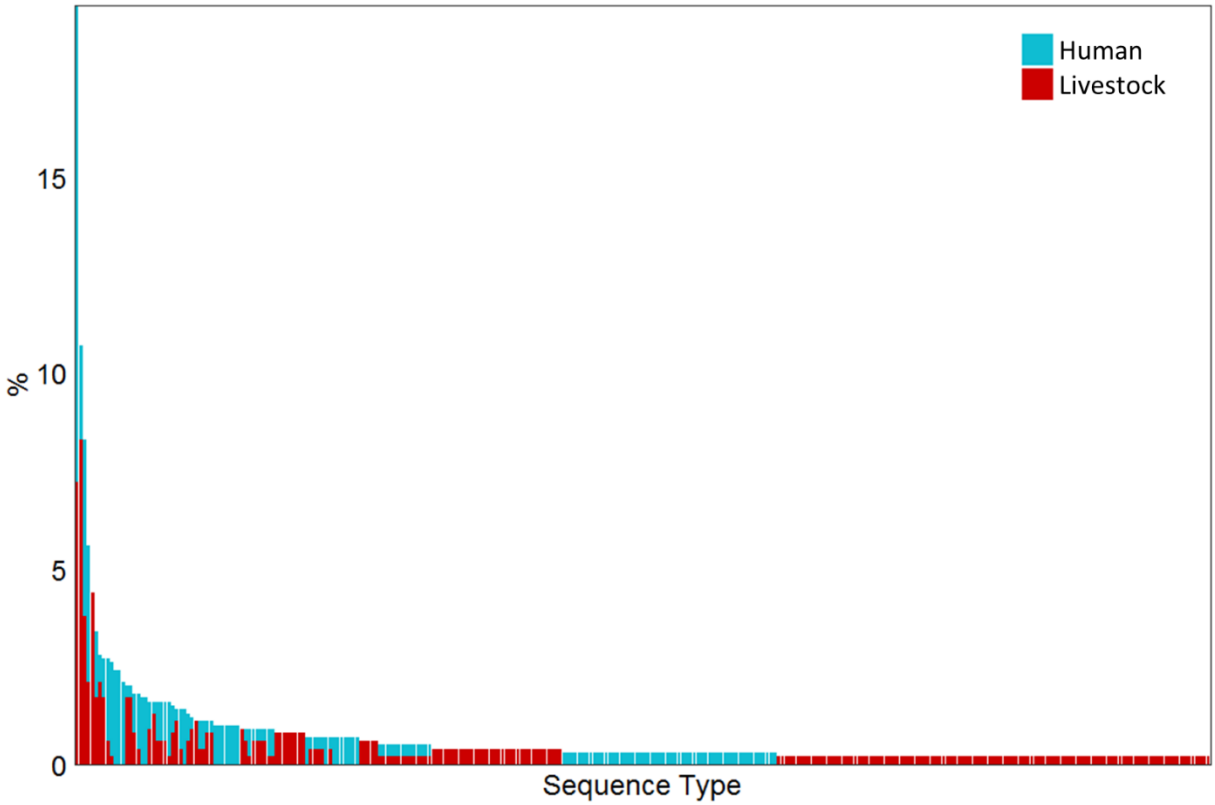

Figure 6.3 Distribution of multi locus sequence types by host group

Of the 296 identified STs, 78 (26%) were unique to humans, 163 (55%) to livestock, and 55 (18%) were found in both human and livestock isolates. The three most common STs associated with human isolates were ST10, ST155 and ST58, while the three most common STs associated with livestock were ST48, ST10 and ST297, although the distribution of STs varied depending on the livestock host. The greatest overlap in STs (absolute counts) between human and livestock isolates occurred in ST10 (12.2% and 7.2% of humans and livestock isolates, respectively), ST48 (2.4% and 8.3% of humans and livestock isolates, respectively) and ST155 (4.5% and 3.8% of humans and livestock isolates, respectively). Across all sequence types the distributions did not significantly differ between human and livestock isolates (p value >0.05 Fisher's Exact test, adjusted for multiple testing). Notably, I identified 5 human isolates belonging to ST 131, a clinically relevant clonal group often associated with dissemination of CTX-M-15 class of ESBL gene.

As determined using goeBURST and a minimum spanning tree analysis (Figure 6.4), the STs were grouped into 37 clonal complexes (CC), and 165 singletons (sequence types that did not cluster with any other published ST), indicating that eBURST might be unreliable in estimating relatedness in this largely diverse *E. coli* population. Two main subpopulations were identified, CC1 and CC2, predominantly associated with ST 48 and ST155 respectively. The two clonal complexes were mostly comprised of human and poultry isolates and accounted for 38% of the isolates, (Figure 6.4).

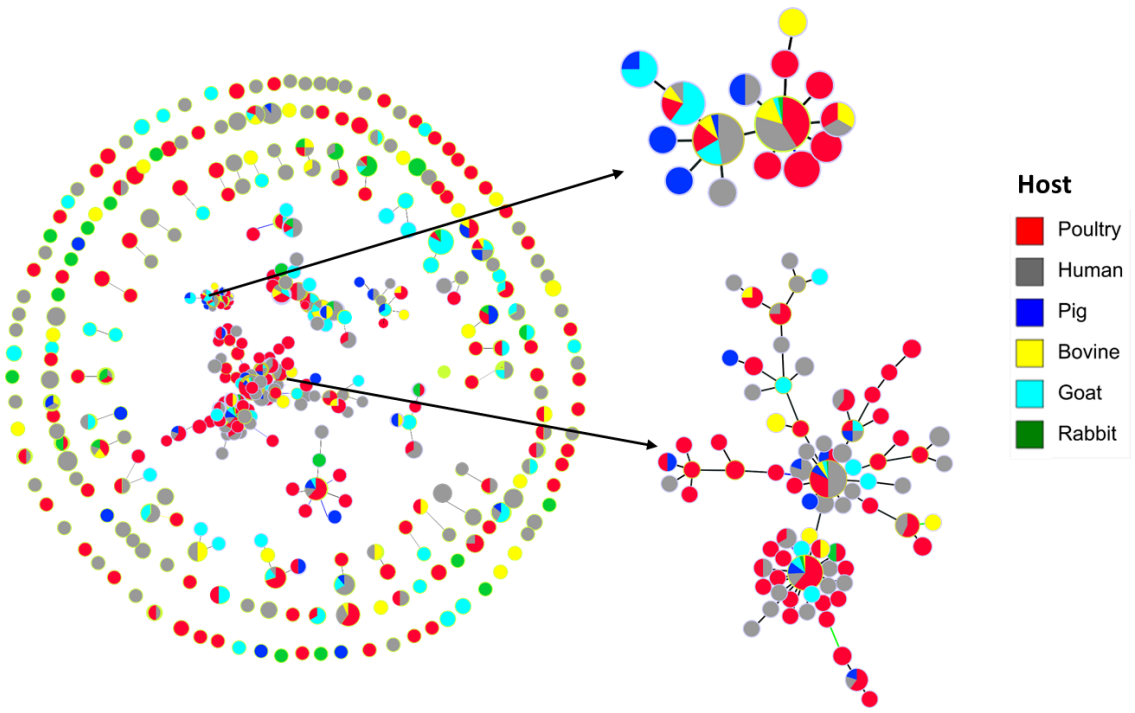

Figure 6.4 Minimum spanning tree reflecting clonal relationships of allelic profiles of a) 869 *E.coli* isolates and the two most common clonal complexes (CC1 and CC2) constructed using goeBURST. STs are symbolized by dots; the size of a dot corresponds to the number of isolates belonging to an ST (major clusters labelled). The colour of each circle represents the source of the strains belonging to that ST. Single locus variants (SLVs) are linked by solid lines. A clonal complex was defined as a group of STs that had ≥ 6 identical alleles.

6.3.3 Genetic differentiation of *E. coli* isolates from humans and livestock

The key study objective was to determine the extent of genetic diversity of commensal bacteria from humans and livestock isolates. To do so, a maximum likelihood tree of the 301 human and 568 livestock genomes, based on 184,786 SNPs in the core gene alignment, was constructed. This analysis revealed a diverse population of *E. coli* population (Figure 6.5) and that genomes from the two categories were intermixed and distributed across the phylogeny, with no evidence of clustering by host group. However, there was evidence of a highly related cluster of goat samples, perhaps denoting a specific goat clone/lineage (Figure 6.5). Isolates involved in this cluster were found in 12 different households and belonged to ST 297.

Next, to gain insight into the allelic diversity among isolates within a sequence type (a commonly used method to infer relatedness), I analysed all pairwise SNP differences between isolates that belonged to sequence type 10 (found in 12.2% and 7.2% of humans and livestock isolates, respectively). Figure D2 in Appendix D shows a histogram of the 2628 pairwise allelic profile comparisons. This analysis revealed that just five pairs (<1% of comparisons) differed by less than 10 SNPs and that the median pairwise distance was 2415 SNPs (range of 1 to 6686 allelic differences), highlighting that most of these isolates were genetically diverse and genetically distant from each other. A visual comparison of isolates belonging to ST10 (Figure 6.5, outer ring, black colour) indicated that the some isolates were separated by long branches indicating deep evolutionary relationships within a sequence type.

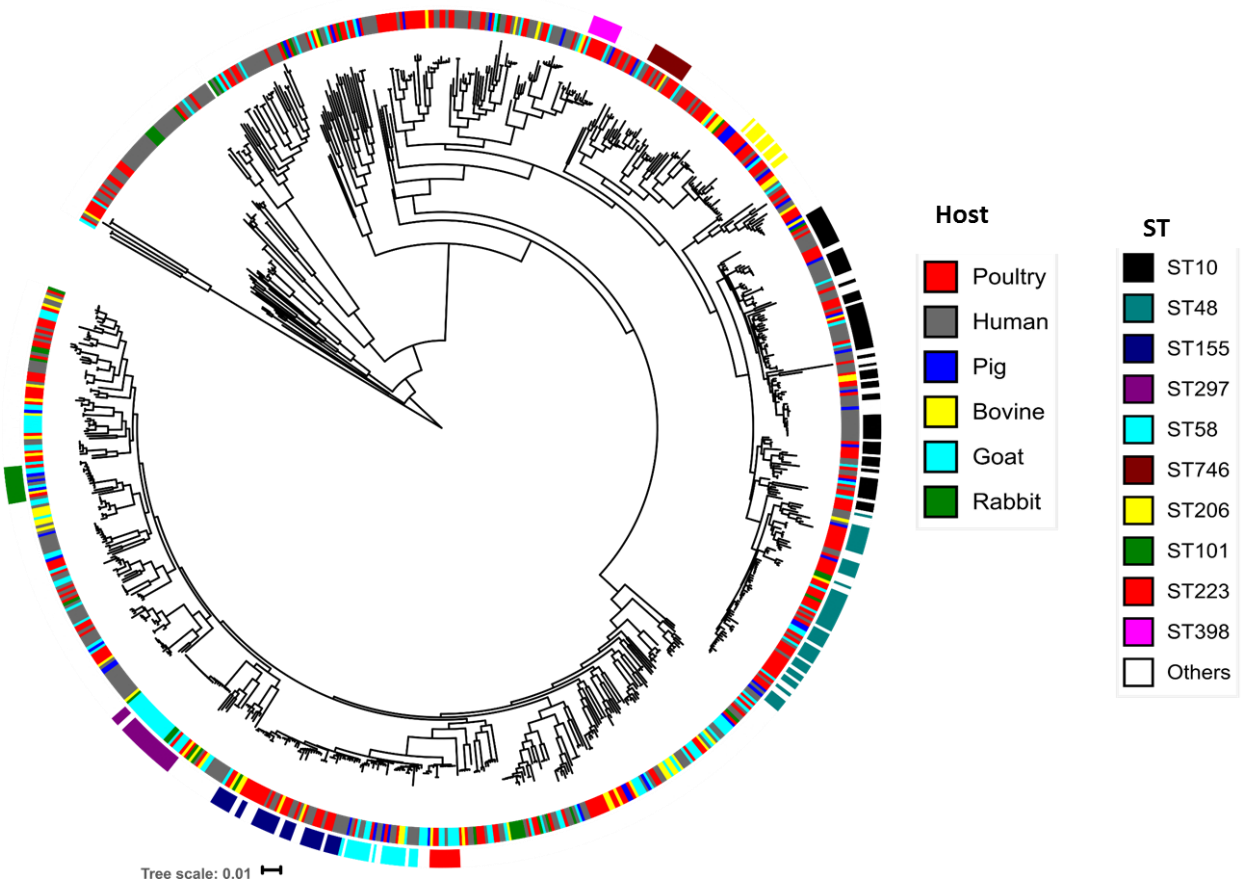

Figure 6.5 Maximum likelihood tree based on SNPs in the core genes of 869 *E. coli* isolates cultured from humans (n=301) and livestock (n=568). Coloured rings indicate, from the centre out, the host of each isolate and the most common sequence type.

6.3.4 Analysis of bacterial sharing of *E. coli* isolates within and between human and livestock species

Pairwise SNP analysis identified 91 isolate pairs that differed by less than or equal to 10 SNPs. The estimated mutation rate for *E. coli* is one SNP/core genome/year (Stoesser et al., 2016), and so the 91 pairs were associated with recent transmission (up to 10 years) within or between human and livestock populations.

To analyse and visualise the relatedness of *E. coli* strains within and between human and livestock populations, a network was developed for isolates classified as genetically related (less than or equal to 10 SNPs different, equivalent to approximately 10 years of evolution). Of the 91 isolate pairs that were closely related, 23 were within humans, 59 within livestock and 9 between humans and livestock. Evidence of *E. coli* relatedness between different livestock species, with the exception of pigs was evident. (Figure 6.6).

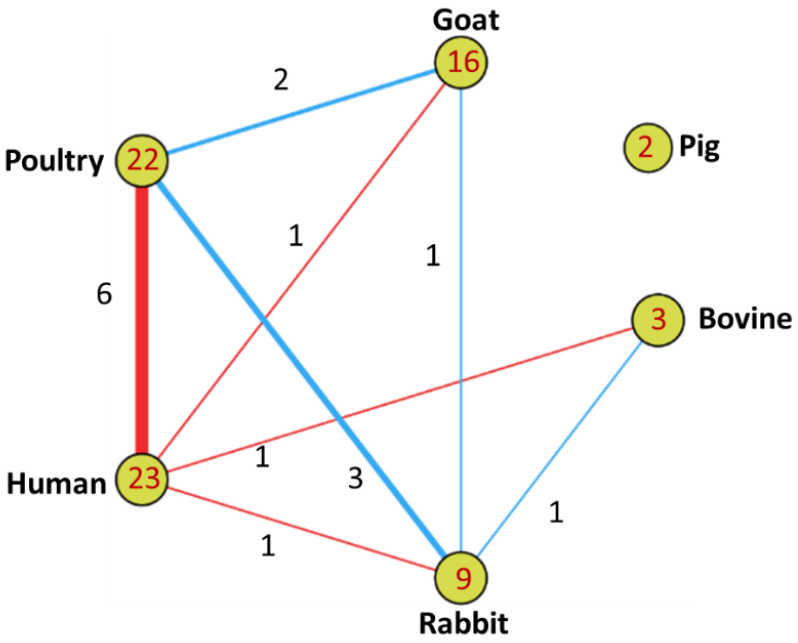

Figure 6.6 Network analysis of *E. coli* isolates from humans and the different livestock populations. Lines are drawn between host groups sharing isolates less than ten SNPs, with arbitrary line lengths. Thickness of lines denotes number of sharing events. Numbers in nodes designate number of sharing within the host group.

Across the 99 study sites, I identified 42 (42.4%) households with at least one sharing event. Of the 42 households, 22 (52.4% of 42) households were involved in livestock-livestock transmission only, 8 (19%) human-human only, 5 (11%) both human-human and livestock-livestock, 4 (9.5%) human-livestock only, 2 (4.8%) both human-livestock and livestock-livestock, and 1 (2.3%) with both within hosts and between hosts sharing.

Next I analysed whether the number of sharing events in a households was dependent on the number of samples collected. Two households (HAR011 and KYL027) had a sharing rate of more than 50% (number of sharing events as a function of number of samples collected). In HARO11, of 20 livestock samples collected, 12 (60% - 6 in poultry and 6 in rabbits) isolates were involved in a sharing event, and could be resolved into 2 clusters. Similarly, in KYL027, of 7 human samples collected, 4 (57%) were involved in sharing and belonged to one cluster.

6.3.4.1 Human-livestock sharing events

The nine sharing events between humans and livestock could be resolved into eight transmission clusters, each involving between 2 and 4 isolates. Of the nine events, six involved poultry, one bovine, rabbit and goat respectively. No isolates from pigs were closely related to human isolates. Isolates in the 9 pairs belonged to seven different STs (ST10, ST48, ST6178, ST58, ST538 ST23 and ST206) and one unknown sequence type. Males were significantly more likely to be involved in human-livestock sharing that females (p=0.003, Fisher's Exact test). Further, 6/9 of persons involved in human-livestock sharing had direct contact with livestock through collecting eggs, slaughter, milking or handling manure, although not significantly significant (p=0.1, Fisher's Exact test) (Table D1 in Appendix D).

The sharing events occurred in 10 household, representing 10% of the sampled households. Of the 10 households with a sharing event, eight had livestock while two did not, hence the two transmission events happened with livestock from neighbouring households. Further, of the two between-household sharing events,

one human-poultry transmission occurred in households located in opposite sides of the city highlighting long range dispersal of bacteria (Figure 6.7).

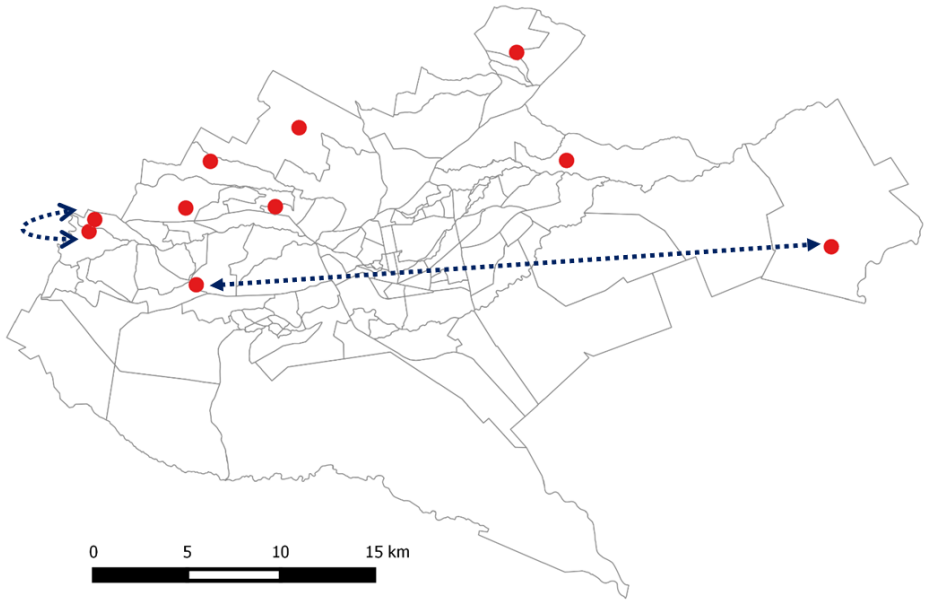

Figure 6.7 Spatial distribution of the 10 households involved in human-livestock clonal sharing. Arrows denote sharing events involving two separate households

6.3.4.2 Human-human sharing

Overall, of 23 human-human sharing events with 0-10 SNPs identified in this dataset, three had 0 SNPs different, six had 1-3 SNPs, and 14 had 4-10 SNPs different. Further, the 23 sharing events could be resolved into 15 transmission clusters. Twenty-two sharing events involved ten different sequence types. No ST was assigned to isolates in one sharing event.

ST678 was identified as the most common sequence involved in sharing (6 of 23 pairs, 26.1%), followed by ST2531 and ST10 (both identified in 4 pairs, 17%). Two sharing events belonged to ST131. Other infrequent sequence types (found in one pair) identified included: ST1136, ST155, ST1611, ST210, ST216, and ST-69 (Figure 6.8).

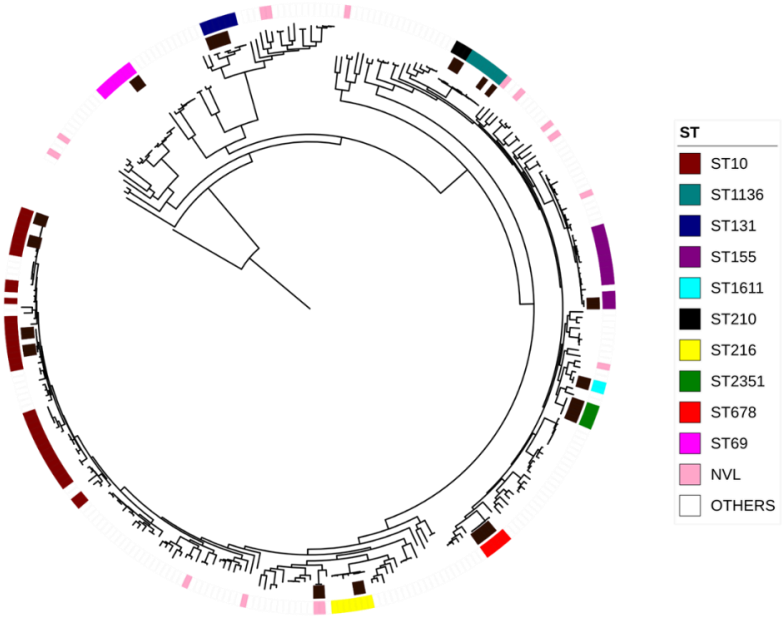

Figure 6.8 Circular maximum-likelihood core-gene phylogenetic tree of *E. coli* isolates from humans only. Inner ring designates the human-human sharing clusters. Phylogenetic tree was reconstructed based on 153352 SNPs in the core genome. Outer ring designates the most common sequence types.

Across the 99 households, 15 households were involved in sharing with most of sharing (18 of the 23 related pairs, 78.2%) happening within households compared to between households (5 sharing events, 21.7%) (Figure 6.9).

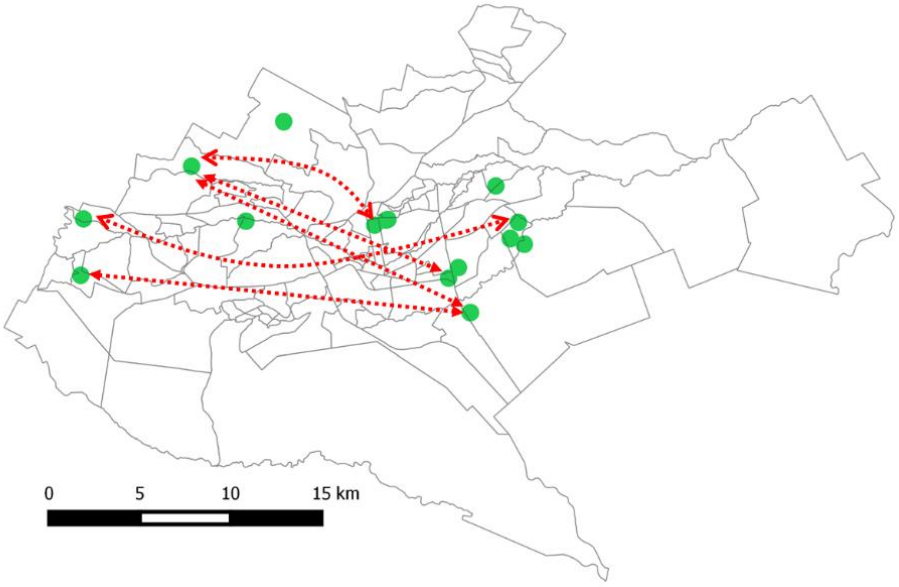

Figure 6.9 Spatial distribution of the 15 households involved in human-human sharing. Arrows denote sharing events involving two separate households.

6.3.4.3 Livestock-livestock sharing

Overall, of 59 livestock-livestock sharing events, three had 0 SNPs different, 19 had 1-3 SNPs, and 137 had 4-10 SNPs different. In 52/59 (88%) of the related pairs, sharing happened within the same livestock species: poultry-poultry (22), goat-goat (16), rabbit-rabbit (9), bovine-bovine (3) and pig-pig (2). The remaining seven pairs happened between different livestock species: poultry-goat (2), poultry-rabbit (3), goat-rabbit (1), and rabbit-bovine (1).

Further, the sharing pairs involved 28 different sequence types, while isolates in two sharing events the sequence type could not be explicitly defined *in silico*. The most common STs involved in sharing were ST297 (7 of 59 pairs, 12%), ST 1196 and ST 4568 (both identified in 6 pairs, 10% respectively) (Figure 6.10, Table D2 in Appendix D).

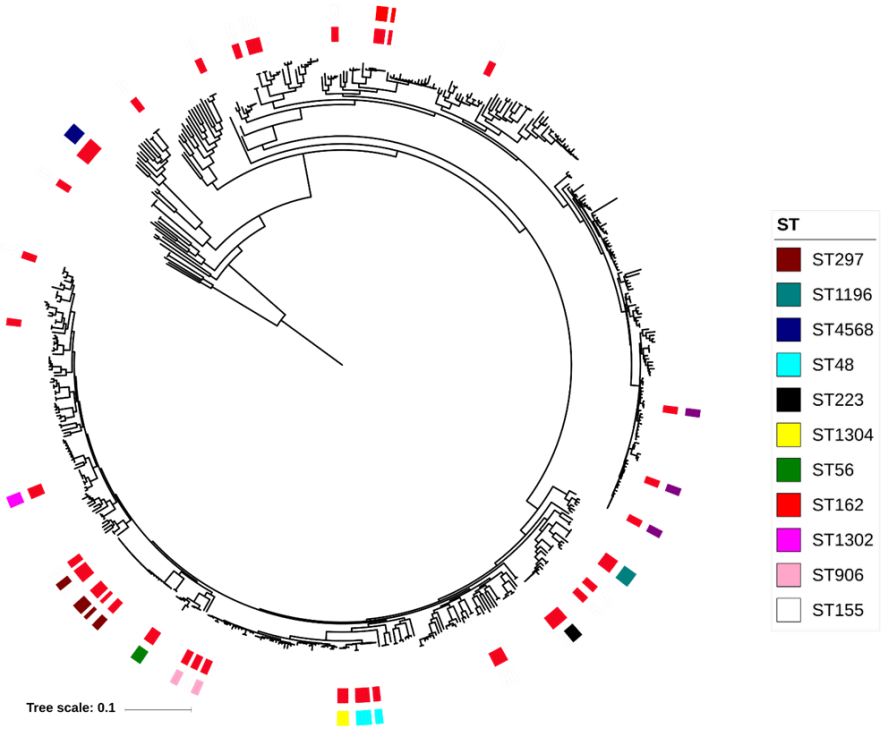

Figure 6.10 Circular maximum-likelihood core-gene phylogenetic tree of *E. coli* isolates from livestock. Inner ring designates the livestock-livestock sharing clusters. Phylogenetic tree was reconstructed based on 358449 SNPs in the core genome. Outer ring designates the most common sequence types.

In 50 (85%) of the 59 related pairs, sharing occurred within households while in 9 (15%) instances transmission occurred between households (Figure 6.11).

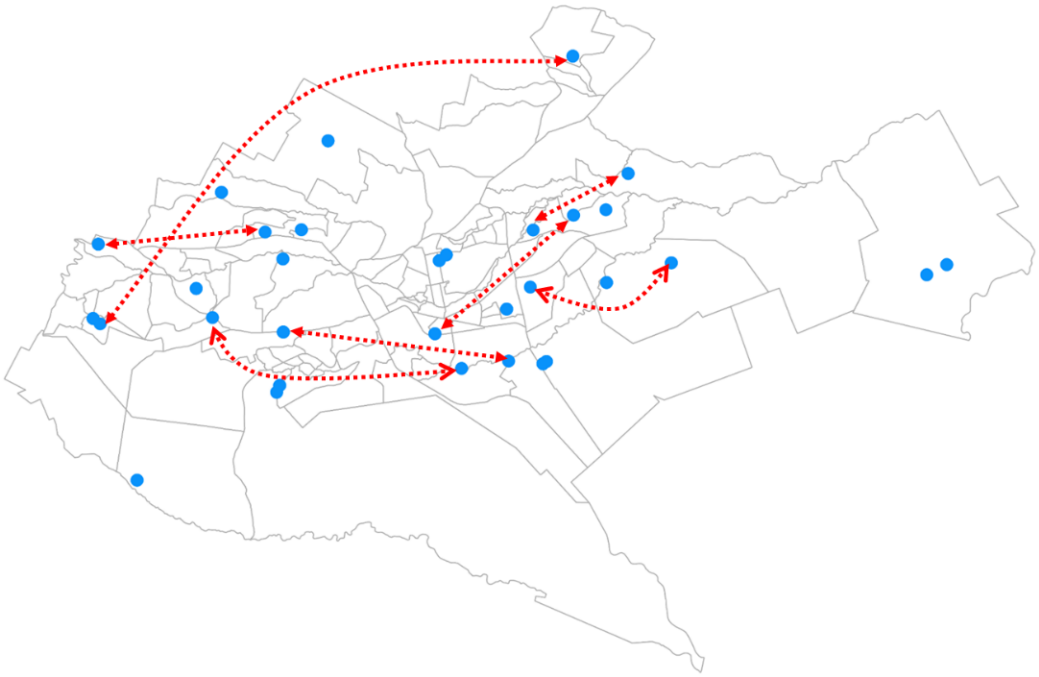

Figure 6.11 Spatial distribution of the 15 households involved in livestock - livestock sharing. Arrows denote sharing events involving two separate households.

6.3.5 Contribution of livestock keeping on diversity of *E. coli* in humans

Here I compared the phylogeny of human isolates with respect to livestock keeping status. This demonstrated that genomes from the two categories were intermixed and distributed across the phylogeny, with no evidence of clustering by livestock ownership (Figure 6.12).

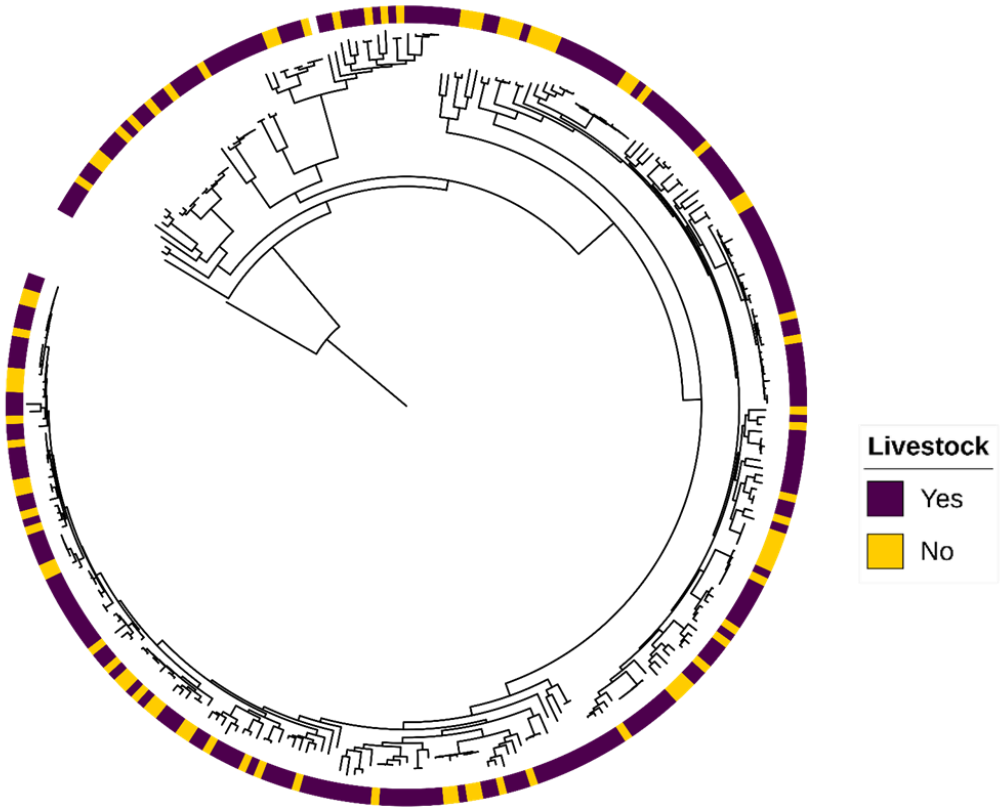

Figure 6.12 Circular maximum-likelihood core-gene phylogenetic tree of *E. coli* isolates from humans. Phylogenetic tree was reconstructed based on 153352 SNPs in the core genome. Inner ring designates the livestock keeping status. Outer ring designates the transmission clusters (<10 core SNPs).

6.4 Discussion

This analysis demonstrates how bacterial whole genome sequencing analysis explicitly embedded within an epidemiologically structured sampling framework can be utilized to investigate the genetic relatedness of *E. coli* from sympatric livestock and humans in a low resource urban setting.

The diversity of *E. coli* in this study was consistent with previous studies (Musicha et al., 2017; Richter et al., 2018; Ludden et al., 2019b) and that, ST10, ST155 and ST48 were the predominant STs. STs 10 and 48 are also the most widespread and diverse within MLST, suggesting long periods of carriage. Five human isolates were found to carry the globally significant clone ST131 with plasmid mediated AMR mechanisms including $bla_{CTX\text{-}M\text{-}15}$ and *aac(6')Ib-cr* conferring an ESBL and fluoroquinolone resistance phenotype respectively (Coque et al., 2008b). This ST is classed as "high-risk" owing to accumulated AMR and proven transmission potential (Johnston et al., 2010).

It is interesting to note that 18% of STs were found in both human and livestock, with the greatest overlap happening in the most common STs. Previous studies that compared isolates from human and livestock sources using MLST indicated that there was evidence of clonal sharing (Leverstein-van Hall et al., 2011; Willemsen et al., 2012; Hu et al., 2013). Analysis of ST10 but using the more discriminatory sequence based analysis indicated that isolates from the two reservoirs were genetically distinct (median pairwise SNPs 2415). Similarly, analysis of the population structure of *E. coli* using goeBURST and phylogroups revealed that the same clonal complex or phylogroup could be found in both human and livestock isolates. These routinely used methods offer advantages in the characterization of acute outbreaks, but their application is limited in non-epidemic situations, in which population structure is more diffuse (Turrientes et al., 2014).

With respect to population structure, there was no evidence of phylogenetic clustering between human and livestock isolates, suggesting that specific

commensal *E. coli* lineages are, in general, not restricted to either human or livestock hosts. This distribution of isolates across both population groups contrast recent studies that indicate that human and livestock isolates are genetically distinct (Ludden et al., 2019b; Nguyen et al., 2019). Conversely, this results indicate a closely related lineage of goat *E. coli* isolates belonging to ST297 and found in 12 households suggesting that the lineage is highly mobile between households, a finding of note for veterinary agencies. It is unclear what factors contribute to the epidemiological success of this clone as the households belonged to independent farmers and not co-located. I speculate that the different farms bought goats from a common source or that the goats shared a great-great-grandmother, with the potential for vertical transmission.

Across the analysed isolates, I detected 91 pairs of *E. coli* isolates that differed by less than 10 pairwise SNP distance based on core gene alignment suggesting sharing within approximately the last ten years within/between these reservoirs. Of the 91 pairs, 82 were within hosts (human-human and livestock-livestock) and nine between human and livestock. Although most (8/9) of the human-livestock sharing events happened in households keeping livestock and that increased contact with livestock was a "risk factor" for sharing, one sharing event happened between livestock and a human not keeping livestock nor with livestock contact. I cannot make direct comparison between the sharing events as this analysis did not incorporate the differential sampling effort which has a direct impact on detection of bacterial sharing. Whilst the frequency of bacterial sharing between humans and livestock was low compared to within host sharing, given the high number of livestock keeping farms in Urban Nairobi (Alarcon et al., 2017), the cumulative burden of bacterial exchange between humans and livestock in the community should not be underestimated.

Within-household sharing was high when compared to between-households and may in part be a result of the greater epidemiological connectivity of human and livestock populations at the household level thus facilitating exchange of

bacteria. Despite the evidence for transmission of *E. coli* between households, the exact mechanism of cross-transmission remains to be established. I speculate that the between-households transmission is probably explained by human/livestock movement, interconnected food supply chains across the city or dispersal of bacterial by peri-domestic wildlife particularly wild birds.

I found that the bacterial community assemblage did not, generally, vary between humans who kept livestock and those who did not. Given the extent of diversity identified in human and livestock populations and that isolates from both populations were intermixed across the phylogeny I hypothesise that there would be minimal evidence of clustering by livestock ownership. Analysis within specific lineages or clonal complexes would be required to disentangle the complex relationship of livestock ownership and acquisition of bacterial clones in humans.

Considering the extent of within-host diversity in commensal *E. coli* by only sequencing a single isolate from each host, it is possible this sampling bottleneck could have underestimated the sharing events hence inadequate to rule out transmission (Stoesser et al., 2015). The decision to sequence a single isolates from each host was made as a cost-based trade-off between the depth of sampling *E. coli* genetic diversity within each individual and the number of unique individuals from which samples could be included. This situation may be less pronounced in clinical settings where endemic circulation of one "outbreak" clone may be more likely.

I underscore the potential role played by other ecological compartments in driving sharing of bacterial strains within and between human and livestock populations. For instance, peri-domestic wildlife and food of animal origin could play a pivotal role in dispersing bacteria across the fragment urban landscapes. In addition, the cross-sectional study design precludes any inferences on the dynamics of bacterial sharing between humans and livestock across Nairobi, hence longitudinal studies are required to understand whether bacterial sharing

within and between host populations is transient or a more permanent colonization.

In conclusion, the combination of whole genome sequencing and detailed epidemiological analysis has revealed an extensive diversity of commensal *E. coli* and evidence of recent sharing of *E. coli* lineages between and within sympatric human and livestock populations. Detection of dissemination of AMR genes independently of host strain rather than sharing of the strain together with the plasmid highlights the need to incorporate plasmid transmission analysis in studies of antimicrobial resistance epidemiology. This study does not, and is not intended to, address the question of directionality of bacterial sharing, in particular human/livestock sharing. To achieve this, future research should consider the synergy between longitudinal sampling of human and animal populations (over time and space), and phylogeographic inferences to investigate host-switching events between humans and livestock hosts (Richardson et al., 2018).

Chapter 7

A cross-sectional survey of practices and knowledge among antimicrobial retailers in Nairobi, Kenya

Work in this chapter has been published in the Journal of Global Health, and a copy of the publication is included in Appendix I.

> The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant
>
> **Alexander Fleming, 1945**

Chapter 7 A cross-sectional survey of practices and knowledge among antimicrobial retailers in Nairobi, Kenya

7.1 Abstract

AMR driven by antimicrobial consumption is a growing global health threat. However, data on antimicrobial consumption patterns in LMICs is sparse. Here, I investigate the patterns of antimicrobial sales in humans and livestock in urban Nairobi, Kenya, and evaluate the level of awareness and common behaviours related to antimicrobial use and AMR amongst human and veterinary pharmacists.

A total of 40 human and 19 veterinary drug store pharmacists were interviewed in Nairobi in 2018 using a standard questionnaire. Data recorded included demographic variables, types of antimicrobials sold, antimicrobial customers, antimicrobial prescribing practices and knowledge of antimicrobial use and AMR.

This study shows that at the retail level, there is a considerable overlap between antimicrobial classes (10/15) sold for use in both human and veterinary medicine. Whilst in this study, clinical training significantly influenced knowledge on issues related to antimicrobial use and AMR and respondents had a relatively adequate level of knowledge about AMR, several inappropriate prescribing practices were identified. For example, most veterinary and human drug stores (100% and 52% respectively) sold antimicrobials without a prescription and noted that customer preference was an important factor when prescribing antimicrobials in half of the drug stores.

Although more research is needed to understand the drivers of antimicrobial consumption in both human and animal populations, these findings highlight the need for immediate strategies to improve prescribing practices across the pharmacists in Nairobi and by extension other LMIC country settings.

7.2 Introduction

As in most cities in LMICs, in urban Nairobi the high incidence of bacterial diseases and antimicrobial resistance in clinical medicine is a major public health challenge (Makobe et al., 2012). In both human and animal populations, antimicrobials are used for both prophylaxis and treatment of infectious diseases and many of the antimicrobials used to treat these infectious diseases are pharmacologically similar. It is estimated that more than half of all antimicrobials (for use in both humans and animals) are purchased without a prescription and used over-the-counter (Brent et al., 2006). There is a paucity of data in Kenya regarding antimicrobial usage at both the national and the regional level, but there have been attempts to assess the consumption of antimicrobials in food producing animals and human health using sales data (Okeke et al., 2005). These studies, based on antimicrobial import data, estimate that, from 1997-2001, consumption of antimicrobials in clinical medicine increased by 4%, with penicillins and fluoroquinolones being the most widely used antimicrobials. Collecting data on antimicrobial use simultaneously in both animals and humans could provide essential data to help disentangle the primary drivers for the development of antimicrobial resistance. Here, I carried out a survey to investigate the patterns of antimicrobial sales in humans and animals in urban Nairobi.

Pharmacists (both human and veterinary) play a pivotal role in enhancing antimicrobial stewardship initiatives, not just by highlighting the AMR problem, but also by influencing crucial prescribing decisions (Broom et al., 2015; Sakeena et al., 2018). To further improve antimicrobial use and antimicrobial stewardship programmes it is important to have an understanding of the knowledge and attitudes towards antimicrobials within different populations such as pharmacists. At present, there has been limited research in understanding pharmacists' knowledge of antimicrobial resistance. Here, I aimed to assess the

level of awareness and common behaviours related to antimicrobial prescribing amongst human and veterinary pharmacists.

7.3 Materials and methods

7.3.1 Study design and setting

Within each of the pre-selected 33 sub-locations described in chapter 2 two community drug stores – one veterinary drug store and one human drug store – were randomly selected and visited. The final distribution of sampled human and veterinary drug stores is shown in Figure 7.1.

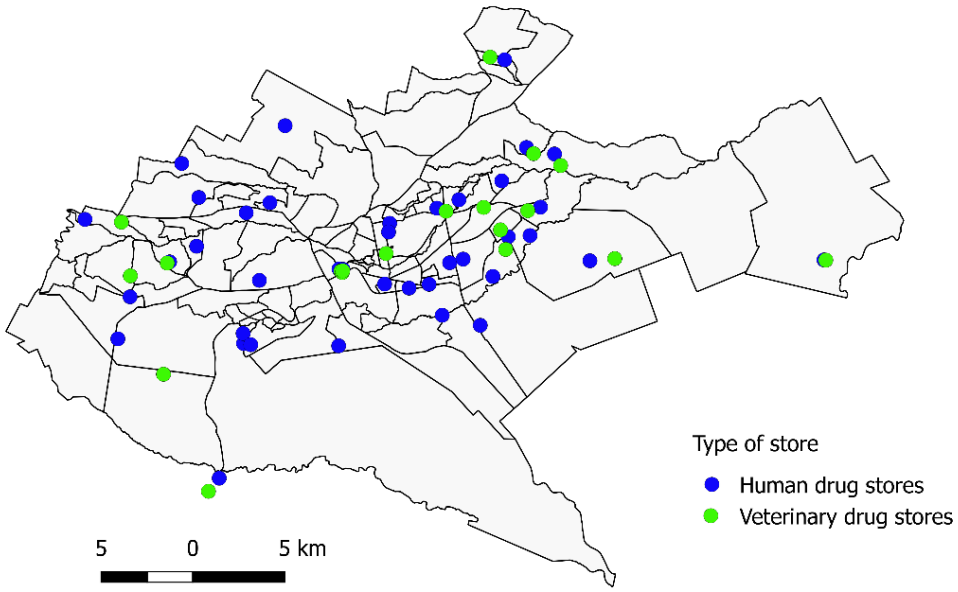

Figure 7.1. Map of Nairobi county indicating locations of study sites.

Community human drug stores are mostly operated by pharmaceutical technicians who are responsible for dispensing antimicrobials, while only a few, mostly large, drug stores have a registered pharmacist (holding a bachelor's degree in pharmacy). Both pharmaceutical technicians and pharmacists are able to sell, but not prescribe antimicrobials in Kenya (Aywak et al., 2017). Veterinary drugs stores are mostly operated by animal health technicians (also referred to as para-veterinarians) with just a few operated by veterinarians. Animal health

technicians are also not allowed to prescribe antimicrobials. All of the above-mentioned groups will have obtained clinical/veterinary training at varying levels. In this study, 'pharmacist' was defined as someone selling antimicrobials in a veterinary or a human drug store irrespective of the level of clinical training.

A draft of the questionnaire was pre-tested with five drug stores (three human and two veterinary), and refined on basis of the feedback from the pre-testing sessions before final dissemination. In each drug store a detailed questionnaire was used to collect data on socio-demographic variables, training on antimicrobial use, types of antimicrobials sold (by class), the four antimicrobial classes most commonly sold, variation in antimicrobial sales, antimicrobial sources, antimicrobial customer characteristics, and antimicrobial prescribing practices. A summary of the collected data is presented in Table E1 in Appendix E. To assess the knowledge of antimicrobial use and AMR amongst the human and veterinary pharmacists a standard questionnaire prepared and used by the World Health Organization was adapted (WHO, 2015a). First, I assessed respondents' knowledge on a number of terms routinely used to describe the problem of antimicrobial resistance. These terms included: antibiotic resistance, superbugs, antimicrobial resistance, AMR, drug resistance and antibiotic-resistant bacteria. Next, respondents were asked about their level of agreement with ten statements describing their knowledge on AMR and potential solutions to antimicrobial resistance. The statements were written on a 5 point Likert scale (Likert, 1932).

7.3.2 Data analysis

Descriptive statistics were prepared for all data including frequencies and percentages for categorical variables (e.g., gender and education level) and means, medians, standard deviations (SDs), quartiles, and ranges for quantitative variables (e.g., number of customers) depending on the distribution of the data. A chi-square or Fisher's exact test using R package stats (Team, 2013) was used to describe differences between proportions of clinical training (present or absent) by type of drug store (human or veterinary) and Mann-Whitney U test to compare range of antimicrobials (number of different antimicrobial classes) in the two types of drug stores.

7.3.3 Prescribing practices

Next, I aimed to describe practices and evaluate the factors associated with drug prescribing amongst human and veterinary pharmacists. To achieve this, I collected data on information provided to customers after purchasing antimicrobials as an indicator of good prescribing practices. This included: whether pharmacists provided customers with information on dosage, directions for use (i.e. completing the prescribed dose), storage instructions, side effects, expiry date and contra-indications (De Vries et al., 1994). The data were then assessed for multicollinearity using the corrplot package (Wei and Simko, 2013) in R to determine if answers to any two or more questions were correlated. There did not appear to be a sufficiently strong correlation between any two questions for any of them to be excluded. To derive a measure of prescribing practices amongst the respondents a composite score (sum of the binary variables, 0/1) from individual indicators of good prescribing practices was developed. I fitted a generalized linear model (GLM) using R package lme4 (Bates et al., 2014) to assess possible influence of type of drug store (human/veterinary), clinical/veterinary training (present or absent), education level (high or low) and range of antimicrobials sold (number of different antimicrobial classes) in the drug store (proxy for store size) on the composite prescribing practices score. Clinical/vet

training (defined as having a degree or diploma in clinical or veterinary medicine) and education level were analysed separately as some pharmacists had received training in disciplines not related to medicine or veterinary studies. P<0·05 was considered statistically significant.

7.3.4 Knowledge on antimicrobial resistance

In order to assess the internal consistency of the ten statements evaluating the level of knowledge on AMR, Cronbach's alpha coefficients were calculated for each statement. Internal consistency is a measure of item-total correlations and reliability of the scale, thus describing the extent to which all items in a test measure the same concept or construct (Tavakol and Dennick, 2011). An unstandardized Cronbach's alpha coefficient of 0.7 or above was considered to demonstrate adequate reliability.

Principal Component Analysis using polychoric correlation (Holgado–Tello et al., 2008) was used to generate a composite index for knowledge score and to investigate clustering of the knowledge statements (Krishnan, 2010). Analyses were performed using the psych package (Revelle, 2017) to conduct PCA (using the principal function) without rotation of axes. Scree plot inspection (Cattell, 1966) and parallel analysis (Cota et al., 1993) were used to choose the optimal model in terms of number of components to retain (Figure E1 in Appendix E, Table E2 in Appendix E).

The scores of the first PCA component were used as measure of knowledge of AMR, and the higher the knowledge score, the higher the implied knowledge of AMR of that respondent. A generalised linear model was used to investigate the possible influence of type of drug store (human or veterinary), clinical training (present or absent), education level (high or low), and range of antimicrobials sold in the drug store (proxy for store size) on the level of knowledge of AMR.

7.4 Results

7.4.1 Demographic data about the respondents

A total of 59 participants were interviewed – 40 from human drug stores and 19 from veterinary drug stores (Table 7.1). Some sub-locations did not have a veterinary drug store as these tend to be located in zones of the city where animals are kept. The median age of participants in both human and veterinary stores was 30 years (range; human, 21-51; livestock, 19-67 years). More than two thirds of participants interviewed in both stores were employees (human, 73% and veterinary, 74%), and the remainder were store owners. Significantly more human pharmacists (90%) than veterinary pharmacists (57%) had undergone some form of clinical training (P=0.01, Fisher's exact test). Additionally, for participants who underwent clinical training, professional development programmes/trainings aimed at continuing education in AMR were an important source of information on antimicrobial stewardship (human pharmacists, 50%; and veterinary pharmacists, 41%).

Table 7.1. Participant demographics and baseline clinical characteristics.

Variable		Human drug stores	Veterinary drug stores
Number individuals		40 (67%)	19 (33%)
Gender	Female	21 (52.5%)	9 (47.4%)
	Male	19 (47.5%)	10 (52.6%)
Highest education level	Primary	0	1 (5.2%)
	Secondary	4 (10%)	5 (26.3%)
	Certificate	4 (10%)	5 (26.3%)
	Diploma	24 (60%)	7 (36.8%)
	Degree	8 (20%)	1 (5.3%)
Role	Owner	11 (27.5%)	5 (26.3%)
	Worker	29 (72.5%)	14 (73.7)
Age (Median)		30	30
Clinical/veterinary training	Present	36 (90%)	11 (57%)
	None	4 (10%)	8 (42.1%)
Source of training on antimicrobial stewardship and AMR	Clinical training only	16 (40%)	3 (15.8%)
	CPD*	20 (50%)	8 (41.1%)
	None	4 (10%)	8 (42.1%)

* Continuing professional development

7.4.2 Antimicrobials available for sale

A total of 15 antimicrobial classes were available for sale in either or both human and veterinary drug stores (Figure 7.2). Two thirds of the antimicrobial classes (10/15) were available in both human and veterinary drug stores while five classes (metronidazole, amphenicols, lincosamides, glycopeptides and carbapenems) were only available in human drug stores. Of the ten overlapping antimicrobial classes, β-lactam/penicillin, tetracycline, sulfonamide, and macrolide antimicrobial classes were found in more than 78% of both types of drug stores. Of note, carbapenems, third and fourth generation-cephalosporins and glycopeptides – antimicrobials restricted to clinical use – were available in 15%, 4% and 3% of human drug stores respectively. Overall, human drug stores had a broader range of antimicrobials available for sale when compared to veterinary stores ($p<0.01$, Mann–Whitney U test) (Figure 7.2).

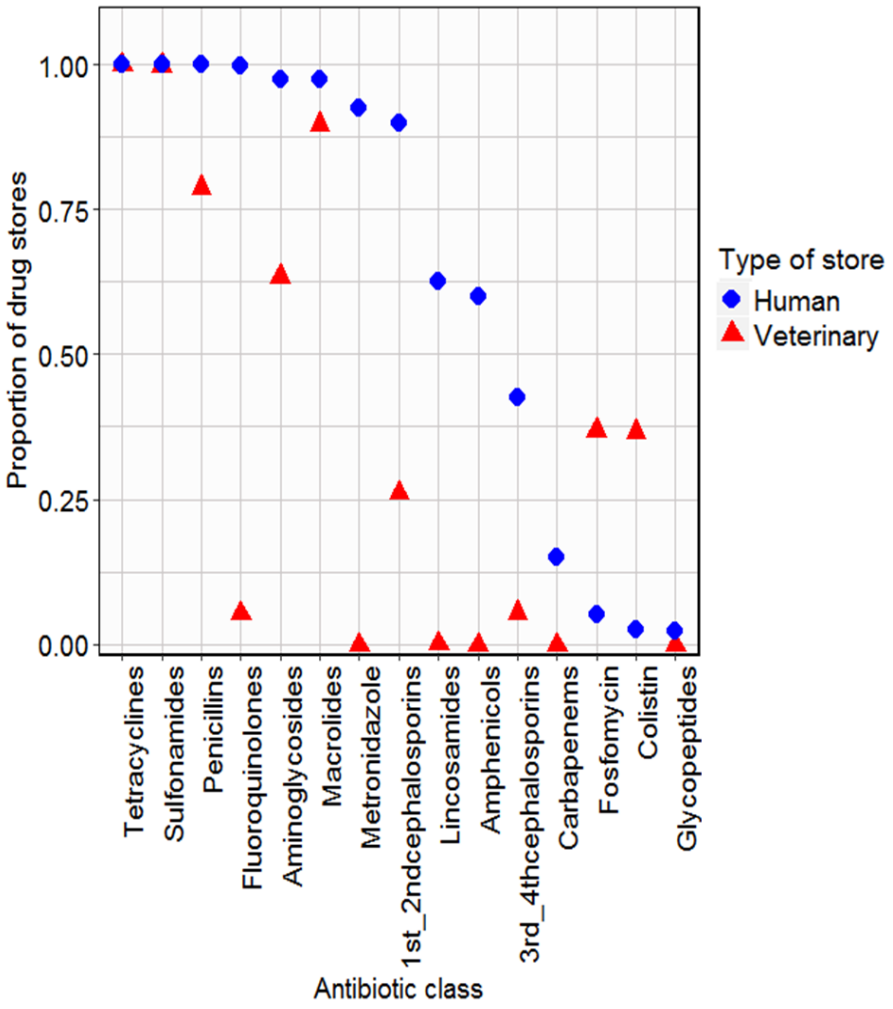

Figure 7.2. Proportion of the 15 antibiotic classes reported in human (n=40) and/or veterinary drug stores (n=19). Data arranged in order of the average proportion of antibiotic classes.

Penicillins, metronidazole, fluoroquinolones, and first and second-generation cephalosporins were reported as being amongst the four most commonly sold antimicrobial classes by the human drug stores in 93%, 65%, 63%, and 43% of the stores respectively. However, among the veterinary drug stores, tetracyclines, sulfonamides, penicillins and macrolides were reported to be amongst the four most commonly sold antimicrobial classes in 79%, 74%, 58%, and 47% of the stores respectively. Tetracyclines and sulfonamides were reported to be amongst the four most commonly purchased antimicrobial classes by poultry farmers in 79% and 90% of the veterinary drug stores respectively. The antimicrobial colistin

was described as being commonly purchased by poultry farmers in 16% of the drugs stores. Sulfonamides, tetracyclines and penicillins were reported to be amongst the four most commonly purchased antimicrobial classes by dairy farmers in 63%, 47% and 52% of drug stores respectively. In 11% of the veterinary drug stores, dairy farmers reportedly purchased first and second-generation cephalosporin intra-mammary tubes to treat mastitis cases. The antimicrobials reported to be amongst the four most commonly purchased antimicrobial classes by pig farmers were penicillins, macrolides and sulphonamides in 37%, 16% and 11% of the veterinary drug stores respectively (Figure 7.3).

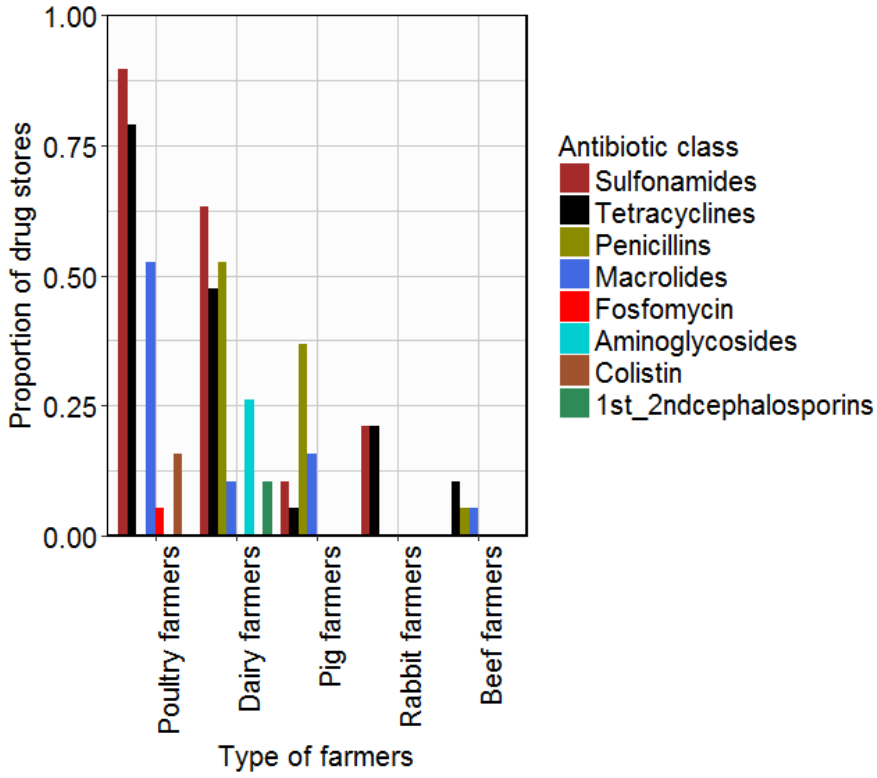

Figure 7.3. Proportion of drug stores reporting the most commonly purchased antibiotics by different types of farmers based on the primary animal on the farm.

Fifty eight percent of human and 42% of veterinary drug stores reported a rise in antimicrobial sales compared to the same period a year earlier. Increased customer demand for antimicrobials was the main driver by 80% and 60% of human and veterinary pharmacists respectively. Wholesale operations (defined as companies that buy drugs in bulk and sell them in smaller quantities to drug stores) were reported as the main provider of antimicrobials to human drug stores (78%). Conversely, distribution companies (defined as corporations that purchase drugs from pharmaceutical companies, store and subsequently distribute to drug stores) were reported as the main provider of antimicrobials to veterinary drug stores (58%).

7.4.3 Antimicrobial customer characteristics

The average daily number of customers purchasing antimicrobials was not significantly different (p=0.2; Mann-Whitney U test) between human drug stores (25 customers, range 2 - 130) and veterinary drug stores (14 customers, range 2 - 113).

Antimicrobials were reportedly prescribed frequently for respiratory tract infections, gastro-intestinal infections, and sore throat in 83%, 65% and 58% of human drug stores respectively. Additional prescriptions were linked to fever, body aches, and skin wounds in 38%, 35% and 13% of human drug stores respectively.

Poultry farmers and veterinary para-professionals were the most frequent buyers of antimicrobials, being reported as customers in 95% and 74% of the veterinary drug stores respectively. Other customers for antimicrobials included: dairy farmers, veterinarians, pig farmers, rabbit famers, and beef farmers in 63%, 58%, 47%, 37%, and 11% of stores.

7.4.4 Knowledge of antimicrobial resistance

More than two-thirds of the respondents in both stores types were aware of the terms 'antibiotic resistance' and 'drug resistance'. By contrast, fewer than half of respondents had heard of the terms 'AMR' and 'super bugs' (Figure 7.4).

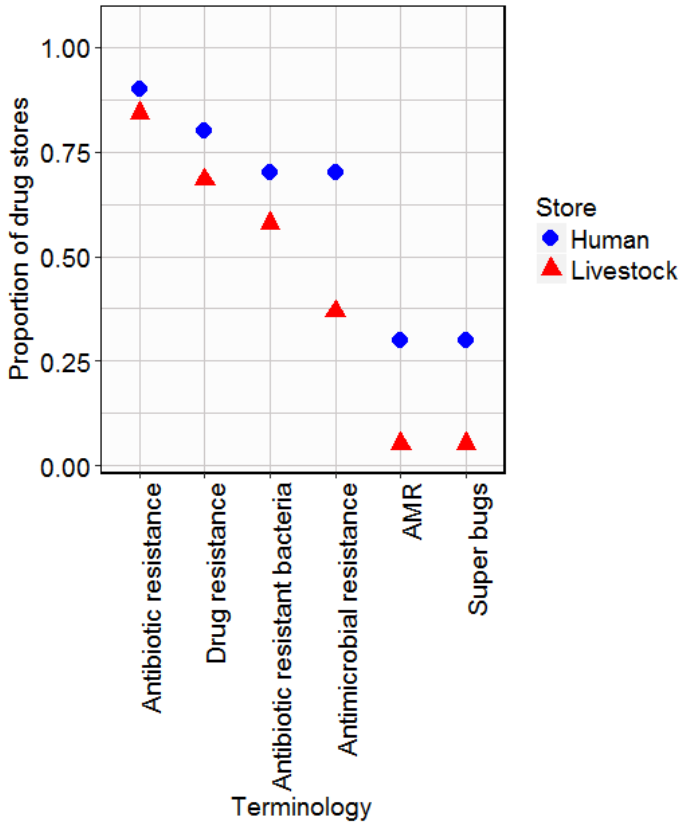

Figure 7.4. Terms used to describe antimicrobial resistance

More than three quarters of the respondents in both store types agreed that the prevalence of drug resistant infections was increasing, and if left unchecked routine medical and surgical procedures would become a much riskier proposition. Likewise, more than 79% in both types of stores recognised that AMR is a problem, and has the potential to affect any country and anyone, including them and/or their families. However, most respondents (>80%) believed that AMR occurs when their body becomes resistant to antimicrobials rather than the bacteria themselves that develop resistance. Similarly, 40% and

53% of human and veterinary respondents respectively suggested that AMR is only a problem for regular consumers of antimicrobials. More than half (52%) of the respondents interviewed responded neutrally or disagreed with the statement that antimicrobial resistant bacteria could be spread from person to person (Table E3 and Figure E2 in Appendix E).

7.4.5 Association between sociodemographic factors and knowledge on AMR

Cronbach's alpha coefficient for the ten "knowledge statements" was 0.74, suggesting an acceptable level of internal consistency and a potential underlying latent construct (Table S2 and Figure S1 in the Online Supplementary Document). The knowledge score (knowledge about AMR) of the respondents had significant positive association with medical/veterinary training (P=0.02), meaning respondents with clinical training had a higher knowledge about AMR than those who had not undergone such training. The level of knowledge about AMR did not differ by store type, education level or range of antimicrobials available in the store ($p > 0.05$) (Table 7.2).

Table 7.2. Results of a multivariable regression examining the influence of sociodemographic factors on knowledge about AMR in a sample of 40 and 19 human and veterinary drug stores respectively.

Variable	Estimate	Standard error	X^2	df	p-value
Veterinary drug store	0.07	0.02	0.3	1	0.59
Range of antimicrobials	-0.01	0.02	0.31	1	0.58
Clinical/veterinary training	0.3	0.14	4.86	1	0.02
High education level	0.04	0.12	0.13	1	0.72

7.4.6 Knowledge and views on potential solutions to AMR

More than 80% of respondents in both store types agreed that people should use antimicrobials only when prescribed by a medical practitioner. Also, more than two thirds of respondents in both store types agreed that reducing antimicrobial use in food animals could help address the problem of antimicrobial resistance. In both store types, respondents agreed on the need for governments and pharmaceutical companies to invest in research and development of new antimicrobials. More than 84% of all respondents agreed that everyone should use antimicrobials prudently, but more than 73% of respondents thought that medical experts would solve the problem of antimicrobial resistance. Hand washing and vaccination of children against infections were supported by more than 94% of respondents in both store types. However, 38% and 26% of respondents in human and veterinary drug stores agreed that there was not much they could do to stop antimicrobial resistance (Table E4 and Figure E3 in Appendix E).

7.4.7 Antimicrobial prescribing practices

Fifty-two per-cent (21/40) of the human drug stores reported that they sold antimicrobials without a prescription while all veterinary drug stores sold antimicrobials without a prescription. Multivariable logistic regression analysis revealed prescribing practices did not vary significantly by clinical training, store type, range of antimicrobials sold and/or education level ($P>0.05$) (Table E5 in Appendix E).

Across both human and veterinary drug stores, the most important factor for prescribing antimicrobials was indication of use – based on symptoms – (in >75% of the stores) , followed by price of the antimicrobial (in >50% of the stores). Of note, 28% and 31% of human and veterinary pharmacists respectively considered customer preference as an important factor when prescribing an antimicrobial.

7.5 Discussion

In this study I aimed to investigate the patterns of antimicrobial sales in humans and animals in a large and rapidly developing city in a LMIC: Nairobi, Kenya. I also evaluated the level of awareness and common behaviours related to antimicrobial use and AMR amongst human and veterinary pharmacists. This study was based on gathering data on antimicrobials stocked in human and veterinary drug stores across the city, where antimicrobial sales were interpreted as representing antimicrobial usage.

This study shows considerable overlap in the antimicrobial classes (10/15) sold for human and animal use in urban Nairobi, with marked variations in the sale of some antimicrobial classes such as cephalosporins and fluoroquinolones – mostly found in human drug stores. This overlap in antimicrobial classes, including of critically important antimicrobials (WHO, 2017a), highlights the need for prudent use of all antimicrobials and continued monitoring and surveillance of antimicrobial usage in LMIC urban settings (Morgan et al., 2011)

The most common symptoms prompting antimicrobial purchase in humans were similar to those reported in other studies, respiratory tract infections and diarrhoeal disease (Okeke et al., 2005; Feikin et al., 2011; Omulo et al., 2017). Broad spectrum β-lactams, fluoroquinolones, first and second generation cephalosporins and metronidazole were the most commonly sold/bought antimicrobials in human drug stores. This finding is consistent with antimicrobial prescription in the community in previous Kenyan studies (Omulo et al., 2017; Mukokinya et al., 2018), in other low income countries such as Uganda (Mbonye et al., 2016), Tanzania (Horumpende et al., 2018a), India (Gandra et al., 2017) and in high income countries such as United Kingdom (Smith et al., 2018) and the USA (Durkin et al., 2018). The finding that WHO-classified highest priority critically important antimicrobial classes such as carbapenems, third and fourth generation cephalosporins, and glycopeptides were sold over the counter and

potentially without prescription in human drugs stores is of public health concern.

In the current study, tetracyclines, sulphonamides, penicillins, and macrolides were the most commonly purchased veterinary antimicrobials and poultry farmers were the major consumers of antimicrobials. Further, my findings indicate that colistin – a drug considered of last resort in human medicine (Liu et al., 2015) – was the antimicrobial of choice amongst poultry farmers in 16% of veterinary drug stores, as has been found in previous studies in other parts of the world (Carrique-Mas et al., 2015; Gondam Kamini et al., 2016; Wongsuvan et al., 2018). Urban livestock are increasingly important, particularly among the low and middle income population bracket in most low resource urban settings (Satterthwaite et al., 2010; McCarron et al., 2015), and antimicrobial usage is a low-cost alternative for comprehensive hygiene and biosafety measures (Robinson et al., 2016b).

Knowledge about antimicrobial resistance among pharmacists has only been studied to a limited extent in LMICs (Wilkinson et al., 2018). Consistent with a recent multi-country survey by the World Health Organization (WHO, 2015a) this survey found that, whilst the majority of the pharmacists have an understanding of the problem of antimicrobial resistance and the effect(s) on public health, they do not fully understand how AMR develops and spreads. Encouragingly, the majority of respondents (>80%) identified several behaviours that can help reduce AMR burden; such as handwashing, antimicrobial stewardship by both doctors and the public, and ensuring children's vaccinations are up-to-date. However, considering their key role in antimicrobial stewardship, the finding that 38% and 26% of human and veterinary pharmacists agreed there was little they could do to stop AMR highlights the need for enhanced involvement of pharmacists in antimicrobial stewardship programs.

Whilst the majority of the pharmacists interviewed have an understanding of the threat posed by AMR to public health, these data highlight the poor quality of community pharmacy practice, most notably the dispensing of antimicrobials without prescriptions and the inclusion of customer preference as an important factor when selling antimicrobials. Antimicrobials were dispensed without prescription in 53% and 100% of the human and veterinary drug stores respectively; a finding consistent with similar studies in Tanzania (92.3%) (Horumpende et al., 2018b), Serbia (47.2%) (Horvat et al., 2017), Ghana (70%) (Donkor et al., 2012), and broadly across the developing world (19-100%) (Morgan et al., 2011). By contrast, a recent study conducted in community pharmacies in Nairobi reported low sale of antimicrobials without prescription (Mukokinya et al., 2018). Part of this difference, however, may be related to the fact that in that study, information was based on just three pharmacies hence not generalizable across the city. In this study, whilst clinical training significantly influenced knowledge on issues related to antimicrobial use and AMR, prescribing practices did not change with levels of clinical training. Considering the complexity of factors contributing to antimicrobial prescribing, including the public's demand for antimicrobials, behavioural and policy interventions could be explored (Meeker et al., 2016). Because many members of the public in most LMICs bypass healthcare facilities and veterinarians in favour of seeking medication at pharmacies, policy makers could consider expanding the role of pharmacists in antimicrobial stewardship initiatives (Wickens et al., 2013; Ministry of Health, 2017).

Restating the particular relevance of training to antimicrobial stewardship measures, the role of enhanced training in antimicrobial prescribing and AMR has been identified in surveys of both medical personnel and the public, both in Kenya and globally (Goff et al., 2017). Results from a recent survey indicate that only 14.1% of clinicians in a national referral and teaching hospital in Kenya had received more than four lectures on antimicrobial stewardship and AMR as part

of their medical training (Genga et al., 2017). To address this challenge, antimicrobial stewardship needs to be integrated in the undergraduate veterinary/medical curriculums and continuing medical/veterinary education programs.

Similar to other studies (WHO, 2015; Sadiq et al., 2018), this findings indicate greater familiarity amongst human and veterinary pharmacists with 'antibiotic resistance' and 'drug resistance' terminologies, and minimal familiarity with 'AMR' and 'superbugs'. This indicates that public health initiatives on antimicrobial stewardship and/or antimicrobial resistance initiatives need to take an evidence-based approach in designing effective communication strategies (Wellcome Trust, 2015; WHO, 2015b).

This is the first study designed to capture the overlapping patterns of antimicrobial sales in humans and livestock in a developing city via an epidemiologically-structured approach. A variety of approaches are available for assessing patterns of antimicrobial use in humans and animals (Queenan et al., 2017). Considering that pharmacies are the primary level of outpatient/veterinary care (consultation, diagnosis, and prescription of antimicrobials) for many urban dwellers in Nairobi, focusing on them provides important insights into the probable antimicrobial usage patterns at the consumer level. Future research would benefit from conducting longitudinal surveys of antimicrobial use in healthcare facilities and the community to better assess trends over time. While, I acknowledge that this study used a relatively small sample size (19 and 40 veterinary and human drug stores respectively), there was minimal heterogeneity in the results obtained. It is important to highlight that although extrapolating antimicrobial consumption from sales data is not ideal, and care will be required when interpreting these results, various other studies have shown that relying on sales data is of direct relevance for initiatives aimed at monitoring global and national antimicrobial patterns (Moulin et al., 2008; Nga et al., 2014). Although

this study focused on pharmacists in urban Nairobi, these results are likely to be relevant to many other developing cities across the world with large income disparity and where livestock are commonly kept in close contact with humans.

7.6 Conclusions

Monitoring and surveillance of antimicrobial use in LMICs is challenging, but vital, as it provides valuable information for public health policy. This study shows that at the retail level in urban Nairobi, there is a considerable overlap between antimicrobial classes available for use in both human and veterinary medicine. Whilst the majority of human and veterinary pharmacists showed high knowledge about antimicrobial use and antimicrobial resistance, inappropriate prescribing practices were noted, highlighting the need for continued education to the pharmacists and the public, about prudent antimicrobial prescribing and use. Although further research is necessary to understand the drivers of antimicrobial consumption in both populations, it is clear that interventions are urgently required to improve prescribing practices across the pharmacists.

Chapter 8

Co-occurrence patterns of antimicrobial resistance genes and distribution of plasmids in *E. coli* isolates

> *Everything is connected to everything else* – There is one ecosphere for all living organisms and what affects one, affects all.
>
> **Barry Commoner, 1971**

Chapter 8 Co-occurrence patterns of antimicrobial resistance genes and distribution of plasmids in *E. coli* isolates

8.1 Abstract

HGT of AMR determinants, often located on plasmids, is considered to be the main reason for the rapid proliferation and spread of AMR. Here, I investigate the co-occurrence patterns of acquired AMR genes and the role of conjugative plasmids on the epidemiology of AMR spread in *E. coli* isolates from sympatric humans (n=315) and livestock (n=594) from 99 households across Nairobi.

These results indicate that in both human and livestock isolates AMR genes were frequently co-located, potentially enabling the acquisition and spread of multi-drug resistance in a single step. The most commonly co-occurring AMR genes were those encoding resistance against aminoglycosides (*strA, strB*) and sulfonamides (*sul2*), and were dispersed across the bacteria phylogeny suggesting they are potentially exchanged across both human and animal populations independent of bacterial transmission. Screening all isolates for plasmid replicon types revealed 31 different plasmid types present in similar proportions in human and livestock isolates with the exception of colBS512, IncB/O/K/Z and p0111, IncHI1B, more common in humans and livestock respectively. ColRNAI, IncFII, and IncFIB, plasmids were the most common (>40%) plasmid replicons identified, and the carriage of plasmid replicons did not differ by host group. AMR genes were significantly associated with plasmid replicons, specifically the *strA, strB, sul2* AMR gene cluster and the ColRNAI, IncQ1 and p0111 plasmid types.

Taken together, by integrating genomic analyses on a large collection of bacterial isolates, I show evidence of AMR gene connections that are potentially co-transferred on conjugative plasmids. These findings provide insights into the co-distribution of plasmid types and AMR genes transmission in *E. coli* in cohabiting human and livestock living community settings in a developing city landscape.

8.2 Introduction

HGT in bacteria leads to acquisition of AMR genes from the 'mobile gene pool', which can then be vertically propagated (Baker et al., 2018a). This aids a rapid evolutionary adaptation of bacterial pathogens to novel ecological niches, such as new hosts, without the reliance upon rare beneficial mutations arising from spontaneous mutation in bacterial populations (Jain et al., 2003).

The importance of HGT in the current global AMR crisis is exemplified by the rapid global dissemination of AMR genes in both clinical and community setting. Studies suggest that this has happened for extended spectrum β-lactamase encoding $bla_{CTX\text{-}M}$ genes, carbapenemase-encoding $bla_{NDM\text{-}1}$ gene and colistin encoding *mcr-1* gene (Cantón et al., 2012; Johnson and Woodford, 2013; Wang et al., 2018). Crucially, horizontal spread of AMR genes among bacteria by conjugative plasmids shape the evolution and spread of resistant 'superbugs' – a serious public health threat (San Millan, 2018). Evidence of HGT of AMR genes (such as identical plasmids in bacterial species within and between different host groups) would be enhanced by strong epidemiological data, however these datasets are lacking.

E. coli is an ideal model for studying the role of HGT owing to its open pan-genome (Lukjancenko et al., 2010) and that it is a commensal in both animals and humans. Urban Nairobi, a rapidly developing city, is an ideal place to analyse understand the impact of HGT in the emergence of antimicrobial resistant bacteria in cohabiting human and livestock populations. Improved genomic analysis based on whole genome sequencing provides an unprecedented level of resolution useful in understanding how and where AMR genes spread across different host populations or niches.

Using WGS, here, I investigate; (i) the co-occurrence of resistance genes, allowing me to identify combinations of genes, and (ii) the role of conjugative plasmids on the epidemiology of AMR spread in *E. coli* isolates obtained from sympatric humans and livestock from 99 households across Nairobi. I investigate the

hypothesis that the constant exchange of AMR genes within and between human and livestock populations is aided by HGT.

Tracking the genetic context of AMR using targeted sampling and the power of WGS will contribute substantially to the understanding of the mechanistic basis of resistance and its dissemination, and by extension the epidemiology of AMR across developing urban landscapes.

8.3 Material and methods

Details on study design, sample collection, bacterial isolation, whole genome sequencing and bioinformatic analysis are presented in chapter 2.

8.3.1 Co-occurrence network of acquired resistance genes

Two approaches were taken to assess relationships among pairs of acquired AMR genes. First, co-occurrence patterns among pairs of acquired AMR genes based on presence/absence data were assessed with the probabilistic approach of Veech (Veech, 2013) with the 'cooccur' package (Griffith et al., 2016). When the probability that two AMR genes would co-occur more or less frequently than observed, if distributed randomly, was < 0.05, that AMR gene pair was considered to have significant positive or negative co-occurrence, respectively. The model calculates the expected frequency of co-occurrence between each pair of AMR genes based on the distribution of one AMR gene being independent of the second one. It then compares the expected frequency to the observed frequency and returns the probability that a lower or higher value of co-occurrence could have been obtained by chance.

Second, for a subset of AMR gene pairs found to have significant positive co-occurrence a network analysis of co-occurring AMR genes was visualized using igraph package in R (Csardi and Nepusz, 2006). Attributable fractions were used to quantify the relationship between the *strA, strB, sul2* gene cluster and the proportion of *tetA* gene using the package epiR (Stevenson et al., 2013).

8.3.2 Role of mobile genetic elements in transmission of AMR genes

Considering the high prevalence of MDR carriage in the isolates (chapter 4), I hypothesised that that this was due to the co-transfer of groups of AMR genes via mobile elements, in particular plasmids. However, determining the genetic context of resistance genes from *de novo* assemblies from short-read sequencing technologies, such as those offered by the Illumina is difficult, because plasmids often contain repeat sequences thus introducing assembly ambiguity (Orlek et al., 2017). Instead, I used plasmid replicon typing as an indicator of plasmid load and diversity. Plasmid replicons as defined in the PlasmidFinder database were identified from read data via the Center for Genomic Epidemiology batch upload platform (identity ≥90%, coverage ≥60%) (Carattoli et al., 2014).

Additionally, I investigated the potential role of previously identified plasmids associated with the most frequent AMR gene cluster (*strA*, *strB* and *sul2*) in carriage and dispersal of AMR genes in these isolates. Following recent evidence of two plasmids (pCERC1 and pCERC2 (Anantham and Hall, 2012)) associated with MDR in *E. coli* obtained from human samples in sub-Saharan Africa (Ingle et al., 2018), including Kenya, I investigated the *E. coli* genomes to evaluate whether the plasmids and AMR genes were mobilised together. The genetic contexts of the AMR genes (*strA*, *strB* and *sul2*) were explored in detail via manual inspection of the SPAdes assembly graphs using Bandage's (Wick et al., 2015) integrated BLAST search (McGinnis and Madden, 2004). For isolates that had the plasmid and the AMR genes located on the same contig I inferred that those genes were moving together on a plasmid. Associations between AMR gene clusters and the two plasmids (pCERC1 and pCERC2) were calculated using Chi Squared tests or Fisher's exact tests in R.

8.3.3 Plasmid carriage by host types

The Mann–Whitney U test was used to compare the carriage of plasmid replicons between humans and livestock. For comparisons of more than two groups, the Kruskal–Wallis test was applied, and statistical differences were corrected for a multiple comparison test using the Bonferroni correction. P values of <0.05 were considered significant.

8.3.4 Procrustes analyses

To investigate the effect of plasmid replicon types on the distribution of acquired AMR genes, I used Procrustes analysis (Jackson, 1995). The AMR genes matrix and plasmid replicon matrix were Hellinger transformed and Jaccard dissimilarities were calculated in R package Vegan (Oksanen et al., 2015). Each dissimilarity matrix was ordinated using Principal Coordinates Analysis (PCoA) using the vegan function 'betadisper' in Vegan (Oksanen et al., 2015). The symmetric Procrustes correlation coefficients between the plasmid replicon and the acquired AMR gene ordinations, were obtained using the 'protest' function in Vegan.

8.3.5 Visualization of AMR gene clusters and plasmid genotypes against a core gene tree

The presence of AMR gene clusters, pCERC-like plasmids and plasmid replicons was plotted as a heatmap against the core genome SNP phylogeny generated in chapter 6 using the plotTree function https://github.com/katholt/plotTree#r-code in R.

8.4 Results

A total of 909 isolates composed of 315 human and 594 livestock isolates (Table 5.1) were whole genome sequenced and screened for known acquired AMR genes.

8.4.1 Antimicrobial resistance genes characterisation

As reported in chapter five, 60 acquired genes known to confer resistance to 9 antimicrobial classes were detected across all isolates at varying abundances. The most common AMR genes were, *sul2* (46%), *strA* (41.1%), *strB* (41.1%), *tetA* (38.3%), and $bla_{TEM\text{-}1B}$ (25%) conferring resistance to sulphonamides, aminoglycosides, tetracyclines and β-lactams respectively.

8.4.2 AMR gene co-occurrence analysis

Probabilistic modelling of acquired AMR genes co-occurrence revealed instances of positive (AMR genes co-occur significantly more frequently than expected), negative (AMR genes co-occur significantly less frequently than expected), and random AMR genes associations (observed frequency of co-occurrence does not significantly depart from expected) (Figure 8.1). Just 264 (14.9%) of the 1770 total AMR gene pair comparisons yielded statistically significant co-occurrence, comprising 243 (13.7%) positive, and 21 (1.2%) negative. 1506 (85.1%) AMR gene pair comparisons had random associations.

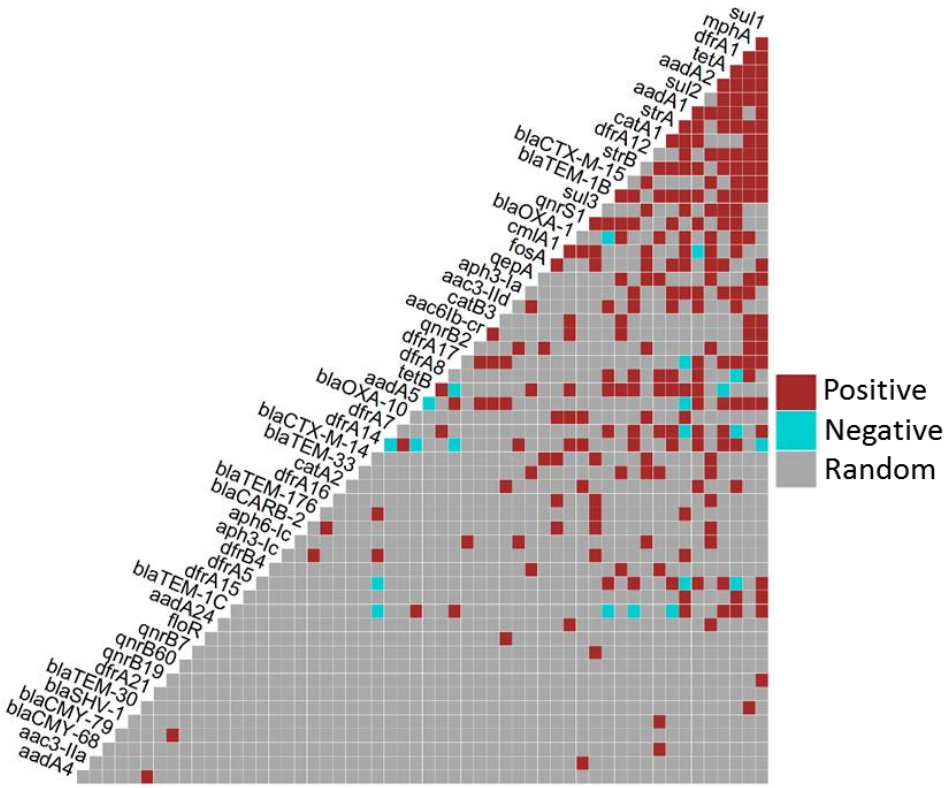

Figure 8.1. Heatmap visualisation showing pairwise associations calculated according to the probability co-occurrence model for 60 acquired AMR genes. Significant positive (negative) associations are displayed where AMR genes co-occurred more (less) frequently than by chance, with an alpha threshold of 0.05. Each tick on the x- and y-axes refers to an AMR gene.

8.4.3 Network analysis

Next, using a co-occurrence network analysis, the most common gene network comprised of *strA*, *strB* and *sul2* AMR genes. This cluster co-occurred in 366 isolates (40.2%): 155 (49.2%) humans, and 211 (35.5%) livestock – 138 (43.9%) poultry, 16 (26.2%) bovines, 25 (50%) pigs, 27 (21.1%) goats and 5 (12.2%) rabbits. The *strA*, *strB* and *sul2* cluster was significantly more likely to be found in human than livestock isolates (X^2 = 8.5, p= 0.003, Chi-squared test). Further, the AMR gene combination significantly co-occurred with *tetA* in 255 (28.1%) isolates (X^2 = 259.51, p<0.001, Chi-squared test), with $bla_{TEM\text{-}1B}$ in 190 isolates (21.1%), and with the combination of *tetA* and $bla_{TEM\text{-}1B}$ in 144 (15.8%) isolates (Figure 8.2).

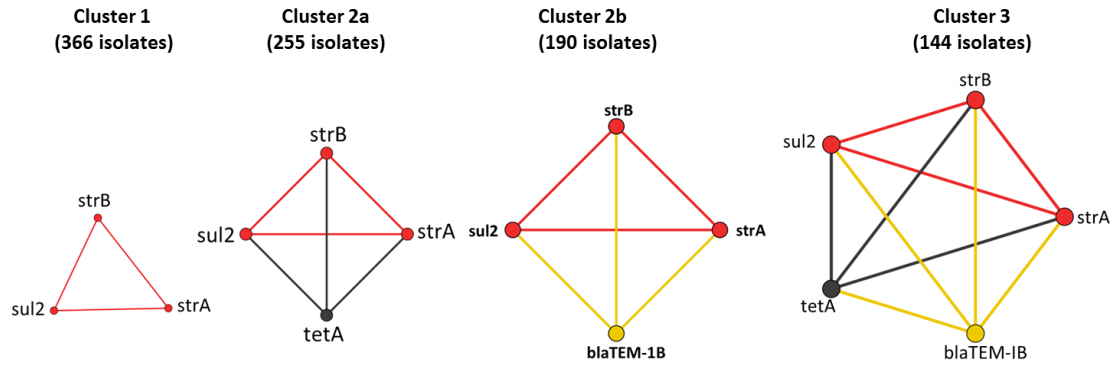

Figure 8.2. The network analysis revealing the statistically significant AMR co-occurrence patterns among human and livestock *E.coli* isolates.

AMR genes making up cluster 1 (*strA, strB, sul2*) and cluster 2a (*strA, strB, sul2, tetA*) were more likely to be found in their respective clusters than outside of it. Conversely, all genes involved in clusters 2b and 3, except $bla_{TEM\text{-}1B}$, were more likely to be found outside the cluster than in it (Table 8.2). Next, I investigated how the presence of *strA*, *strB*, *sul2* genes accounted for the presence of *tetA* (cluster 2a) and $bla_{TEM\text{-}1B}$ (cluster 2b). I found that 76% and 86.7% of *tetA* and $bla_{TEM\text{-}1B}$ occurrences respectively were attributable to *strA*, *strB*, *sul2* genes.

Table 8.1. Proportion of the AMR genes occurring as part of the three clusters.

	Cluster 1 (*strA, strB, sul2*)	**Cluster 2a (*strA, strB, sul2, tetA*)**	**Cluster 2b (*strA, strB, sul2,* $bla_{TEM\text{-}1B}$)**	**Cluster 3 (*strA, strB, sul2, tetA,* $bla_{TEM\text{-}1B}$)**
*strA**	96.3 %	67.1%	50%	37.9%
*strB**	96.3%	67.1%	50%	37.9%
sul2	86.5%	60.3%	44.9%	34%
tetA	-	73.9%	-	41.7%
$bla_{TEM\text{-}1B}$	-	-	83.7%	63.4%

**strA* and *strB* co-occur always

8.4.4 Investigation of mobile genetic elements associated with transfer of AMR genes

As reconstructing the genetic context of acquired AMR genes is generally not possible using short-read sequence data, I analysed the distribution of two plasmids (pCERC1 and pCERC2) previously found to mobilize the *strA, strB, sul2* gene cluster (Ingle et al., 2018). The combination of genes in cluster 1 and the pCERC1 plasmid was found in 62 (6.8%) isolates ($p<0.001$, Fisher's Exact Test) – 21 (6.7%) human and 41 (6.9%) livestock isolates: 35 (11%) poultry, 3 (4.9%) bovines, 1 (0.8%) goat, 1 (2.4%) rabbit and 1 (2%) pig respectively. The combination of the cluster 1 genes and the pCERC2 plasmid was found in 27 (2.9%) isolates ($p<0.001$, Fisher's Exact Test) – 14 (4.4%) human and 13 (2.2%) livestock isolates: 8 (2.5%) poultry, 2 (3.3%) bovines and 3 (6%) pigs. The combination of cluster 1 and both plasmids (pCERC1 and pCERC2) was found in just 6 isolates – 3 (1%) human and 3 (0.5%) livestock. The occurrence of the pCERC-like plasmids together with AMR genes in either cluster 2 (a and b) or cluster 3 was infrequent (less than 2%). Next, I tested the hypothesis that the *strA, strB, sul2* gene cluster could occur independently of the two plasmids. This analysis indicated that the 3 gene cluster remained significant in the absence of the two plasmids ($p<0.01$, Fisher Exact Test).

I further examined the distribution of the AMR gene clusters and the pCERC plasmids on the bacterial core genome phylogeny. Overall, my results revealed that the AMR gene clusters and the pCERC plasmids were interspersed across the phylogeny, indicating extensive dispersal across the bacterial population (Figure 8.3).

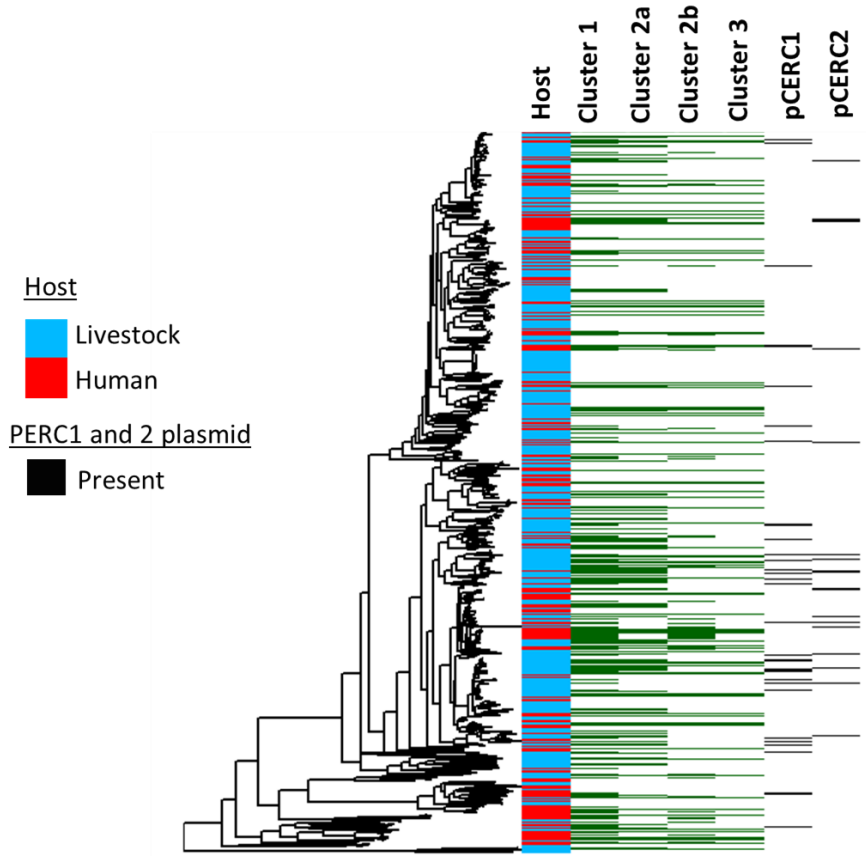

Figure 8.3. Maximum likelihood tree based on SNPs in the core genes of *E. coli* isolates cultured from humans and livestock annotated with host (red, human; blue, livestock), AMR gene clusters (cluster 1 - *strA, strB, sul2*; cluster 2a - *strA, strB, sul2, tetA*; cluster 2b - *strA, strB, sul2, bla*$_{TEM-1B}$; cluster 3 - *strA, strB, sul2, tetA, bla*$_{TEM-1B}$), pCERC1 plasmid and pCERC2 plasmid.

In some genomes, the plasmid sequences could be fully resolved, indicating the location of the AMR gene clusters (Figure 8.4). For example, *E. coli* isolates TMP021231 carried a *dfrA14* gene within a *strA, strB, sul2* cluster on a pCERC1 plasmid, while isolate TMP018919 encoded the cluster on a pCERC2-like plasmid.

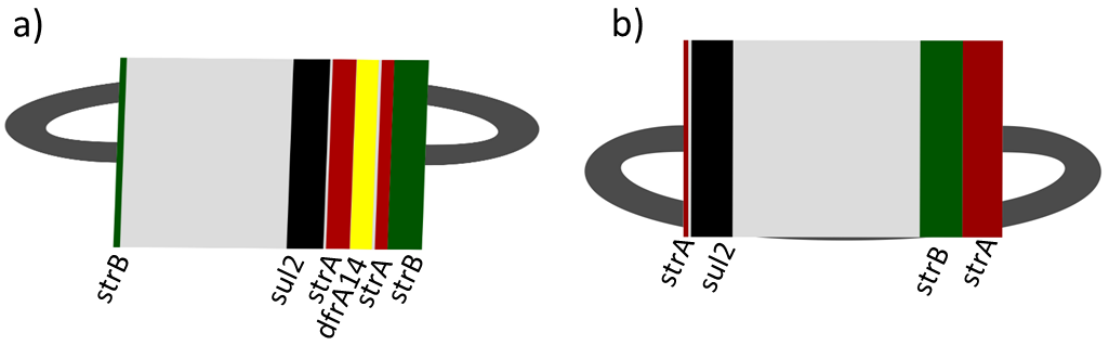

Figure 8.4. Assembly graph for two *E. coli* isolates, TMP021231 and TMP018919, indicating the AMR gene arrangement in (a) pCERC1 and (b) pCERC2 plasmids previously found in *E. coli* isolates. Colour blocks indicate BLAST hits to AMR genes as labelled. Assembly graphs were visualised in Bandage.

8.4.5 Distribution of plasmid replicons

Screening against PlasmidFinder revealed 31 plasmid replicon types in 831/909 (91.4%) *E. coli* isolates. The most common plasmid type across all isolates were ColRNAI (59.8%), IncFII (44.5%) and IncFIB (43.6%) (Figure 8.5). The remaining replicon types were found in ≤15% of the genomes. The distribution of 26/31 (83.4%) replicons did not differ by host, with the exceptions being ColBS512, IncB/O/K/Z and IncQ1, which were significantly more common in humans than in livestock and p0111 and IncHI1B more common in livestock than in humans ($p<0.01$,Fisher Exact Test).

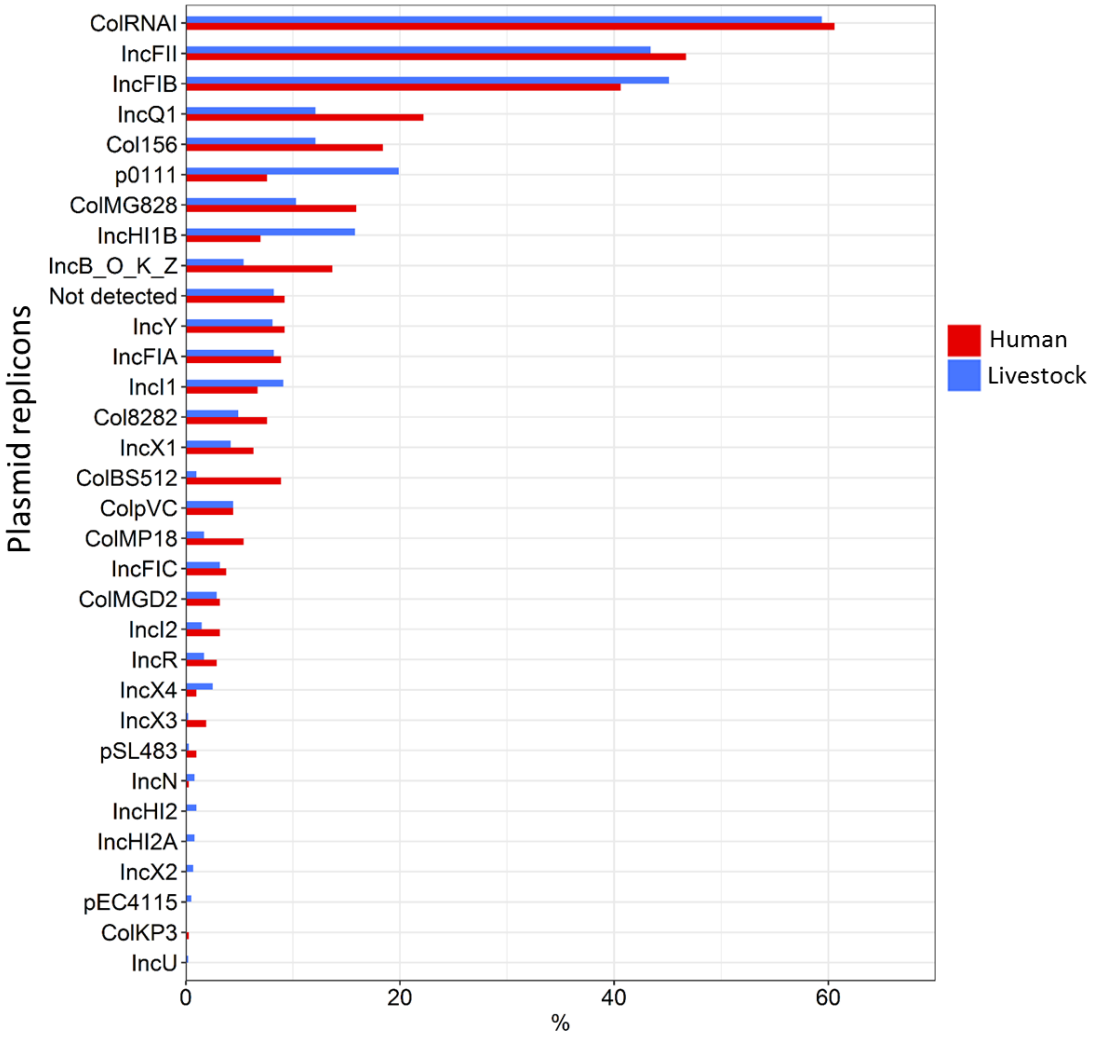

Figure 8.5. Distribution of plasmid replicons in 315 humans and 594 livestock *E. coli* isolates.

8.4.6 Carriage of plasmid replicons by host

Overall, the mean plasmid carriage was three unique matching plasmid replicons identified per isolate, however, carriage did not significantly differ between human (mean 3.08) and livestock (mean 2.81) isolates ($p>0.05$, Man-Whitney U test) (Figure 8.6a). Similarly, when compared to the different livestock groups, human carriage of replicon types was not significantly different from poultry, pigs, bovines and goats ($p>0.05$, Kruskal Wallis, Bonferroni correction). Conversely, human isolates had a higher carriage when compared to rabbit isolates ($p=0.008$, Kruskal Wallis, Bonferroni correction) (Figure 8.6b).

More than one replicon type was found in 698 (76.7%) isolates, and in 289 of these isolates a combination of replicon types IncFIB and IncFII was detected – the most common plasmid replicon co-occurrence ($p<0.001$).

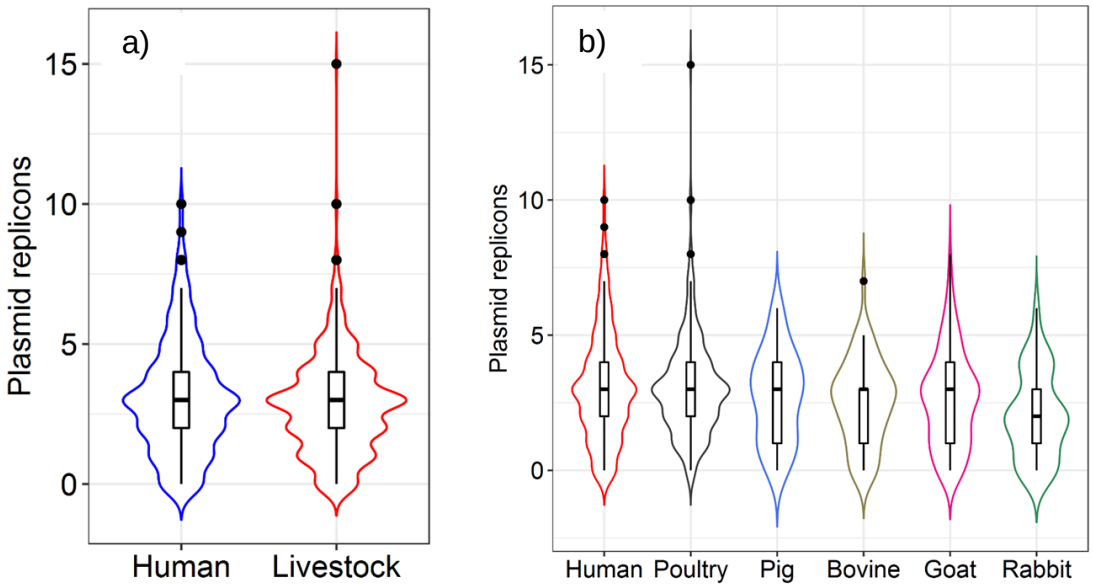

Figure 8.6. Number of plasmid replicons per isolate in a) humans and livestock, b) humans and the different livestock groups. P values were calculated using the Wilcoxon test and Kruskal Wallis. Numbers indicate plasmid replicon diversity within each group.

8.4.7 Association of plasmid replicons with AMR genes and sequence types

Procrustes analysis was used to investigate whether plasmid replicons correlated with the AMR gene composition based on the PCoA of both the AMR genes and plasmid replicons. The analysis revealed that the AMR gene distribution correlated significantly with the plasmid replicon composition, (P = 0.001, 999 permutations, although with low correlation coefficients (correlation: 0.33)). Hence, isolates with similar plasmid replicon compositions tended to have similar AMR gene compositions. Procrustes analyses, however, cannot illustrate the detailed relations between the specific AMR genes and plasmid replicons.

Analysis of the distribution of AMR among plasmid replicon types indicated that the majority of the AMR genes co-occurred with ColRNAI, IncFII, IncFIB, IncQ1 and p0111 plasmids (Figure 8.7, Table F1 in Appendix F), and that IncFII and IncFIB replicons were associated with the same AMR genes, suggesting that these two replicons were potentially on one plasmid. The *strA, strB, sul2* AMR gene cluster (and with *tetA*) was significantly associated with ColRNAI, IncQ1 and p0111 plasmid backbones ($p<0.001$, Chi squared test).

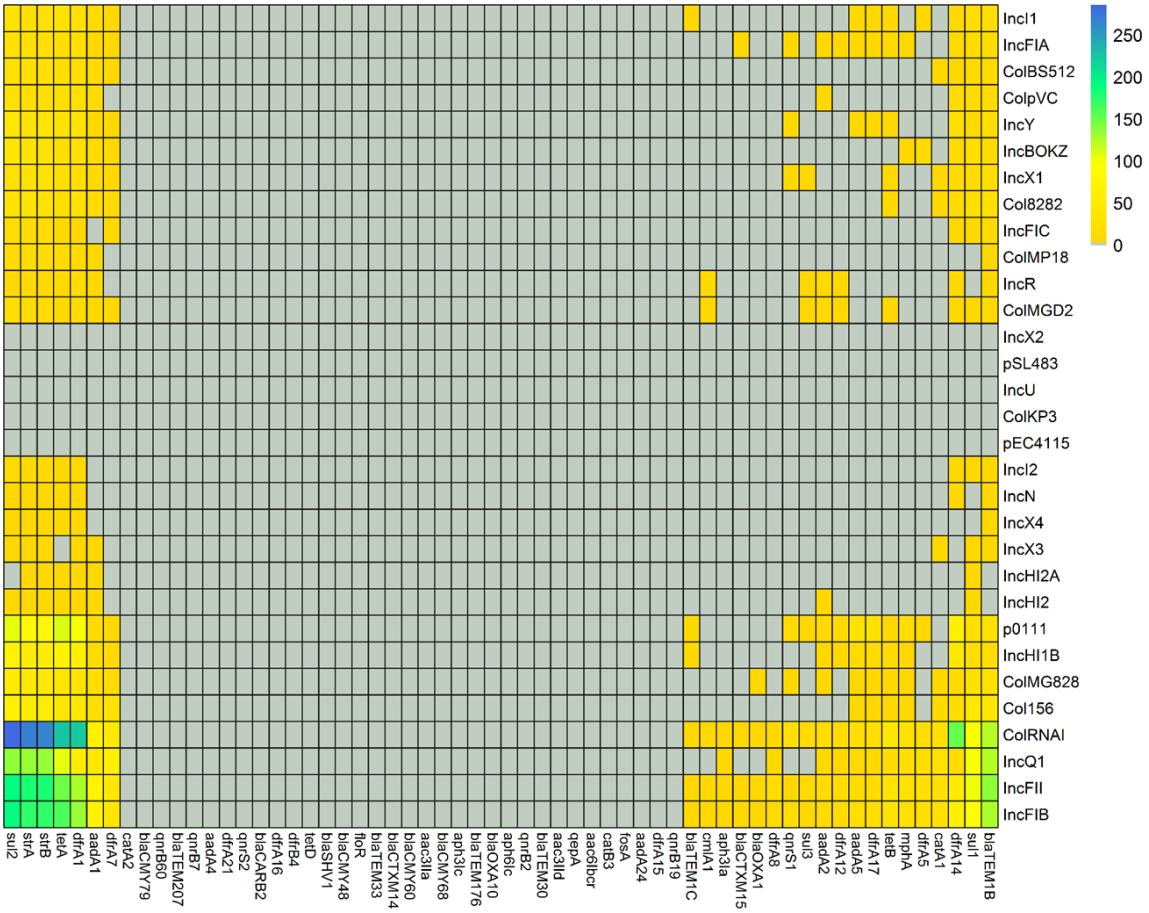

Figure 8.7. A heatmap illustration of associations between plasmid replicon and acquired AMR genes. Different colours represents the strength of the relationship: blue/green - strong, yellow - weak, grey - no association. The scale bar at the right indicates the strength of the associations.

Analysing the association between sequence types and the most common plasmids replicons (present in at least 10 isolates), my results suggest that, overall, plasmid backbones were associated with a variety of sequence types. The three most common replicon types (ColRNAI, IncFII and IncFIB) appeared to be strongly associated with the most common sequence types (ST48, ST155, and ST10) (Figure F1 in Appendix F). Mapping plasmid replicons on the core genome phylogeny revealed that plasmid types were intermixed and widely distributed across the phylogeny (Figure 8.8).

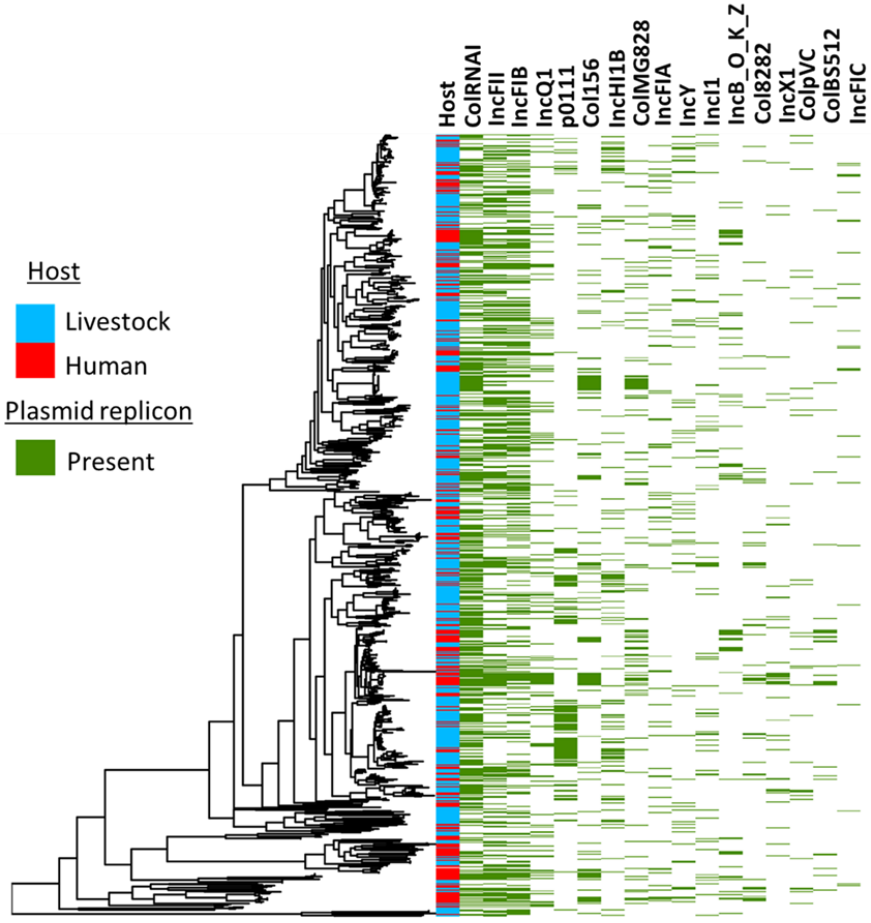

Figure 8.8. Maximum likelihood tree based on SNPs in the core genes of *E. coli* isolates cultured from humans and livestock annotated with host (red, human; blue, livestock), and plasmid replicon types (plasmid replicon columns: green, present; white, absent).

8.5 Discussion

In this study, I investigated the patterns of AMR gene co-occurrence and the distribution of conjugative plasmids. The results provide insight into the role of HGT on the dissemination of acquired AMR genes across host populations.

In agreement with my data relating to a tetracycline-sulfonamide-trimethoprim cluster in the AMR phenotypes (chapter 4), the AMR gene co-occurrence analysis revealed clusters conferring resistance to the three antimicrobial classes, and in addition to β-lactams and aminoglycosides. The AMR gene clusters were ubiquitous across both human and animal isolates highlighting widespread dispersal across both populations. The *strA, strB, sul2* gene cluster (and in combination with other genes) has been identified in previous studies (Yau et al., 2010; Wang et al., 2015; Ingle et al., 2018), and its distribution is likely to be attributed to, (i) co-selection for resistance to the commonly used aminoglycosides and sulfonamides in both human and animal medicine in Nairobi (chapter 7), (ii) maintenance of the gene cluster in the *E. coli* population by a conjugative element. I could not distinguish between hypotheses using this data. Further, the AMR gene clusters were distributed across phylogenetically diverse isolates and involved multiple sequence types indicating that they have become widely established and persistent within *E. coli* populations favoured by selective pressures.

In this study, determining the genetic context of resistance genes and the linked mobile genetic elements from the short-read sequence data was not possible. However, there was evidence of pCERC-like plasmids (pCERC1 and pCERC2) which encode resistance genes mainly to aminoglycosides, sulphonamides, trimethoprim, and infrequently to other antimicrobial classes. The pCERC-like plasmids, have been reported in *E. coli* and *Salmonella* spp. in many countries including Kenya (Anantham and Hall, 2012; Ingle et al., 2018), indicating a global distribution. The *strA, strB, sul2* gene cluster was significantly associated with the pCERC-like plasmids, however, the gene cluster remained significant even in the

absence of the two plasmids. This suggests that the two plasmids did not fully account for the AMR gene clustering, but instead, other mobile genetics elements such as integrons and transposons could be playing an additional role in the occurrence of the gene cluster. In particular, the transposon *Tn6029* has been identified in previous studies to be playing an important role in the dissemination of the *sul2, strA, strB* gene cluster (Chowdhury et al., 2015; Ingle et al., 2018). This finding is a proof of principle for the association between gene clusters and specific plasmids. However, it does not provide a comprehensive explanation for the clustering observed, most of which seems unrelated to those two plasmids.

In agreement with a previous study in Tanzania (Madoshi et al., 2016), in this study, ColRNAI and IncF (IncFII, IncFIB) replicons were the most abundant plasmid types in both human and livestock *E. coli* isolates. ColRNAI, IncF (IncFII, IncFIB) replicons encoding multiple antimicrobial resistance genes have been reported widely in *E. coli* and other Enterobacteriaceae from human and animal samples (Carattoli, 2009; Carattoli et al., 2014; Carroll et al., 2017; Mohsin et al., 2017). Further, the association between AMR genes and plasmid replicons reported in this study, specifically the *strA, strB, sul2* AMR gene cluster and ColRNAI, IncQ1 and p0111, does suggest that plasmids may have a key role in harbouring and disseminating AMR genes. The occurrence of these ubiquitous AMR-plasmids may be linked to positive selection exerted by the high antimicrobial use in both human and animal populations across the urban landscape and are potentially frequent disseminated across both host groups.

Despite the common use of replicon typing in characterising plasmids, a major shortcoming of replicon typing is that individual plasmids can contain multiple replicons, complicating classification, and that it inherently misses new plasmid types (Orlek et al., 2017). To fully characterise the genetic context of AMR genes, further research using long-read sequencing could resolve plasmid structures (George et al., 2017).

In this study I investigated the potential role of horizontal gene transfer in *E. coli* isolates from epidemiologically linked human and livestock populations. Screening of all bacterial genomes revealed clinically relevant connections between resistance genes, widespread in both human and animal populations. The AMR gene connections detected here were associated with previously described plasmids (pCERC1 and pCERC2) and plasmid replicons (proxy for conjugative plasmids) suggesting the existence of a reservoir of resistance genes that is highly mobile. Co-located AMR genes, pCERC-like plasmids and plasmid replicons were ubiquitous and dispersed across the bacterial phylogeny suggesting they are potentially exchanged across both human and animal populations independent of bacterial transmission. Future studies investigating the driving factors associated with accessory gene cassettes transfer and plasmid spreading will contribute substantially to the understanding of the mechanistic basis of resistance and its dissemination.

Chapter 9

Thesis summary and discussion

> Antoine Magnan, a French zoologist, in 1934 made some very careful studies of bumblebee flight and came to the conclusion that bumblebees cannot fly at all! Fortunately, the bumblebees never heard this bit of news and so went on flying as usual
>
> **Ross E. Hutchins, 1968**

Chapter 9 Thesis summary and discussion

Increasing levels of antimicrobial resistance represent a public health and economic threat on a global scale (WHO, 2015b), and the role of livestock in the rising levels of AMR bacteria has been the subject of much speculation. The interplay of AMR transmission between people and livestock is likely to be most evident in LMICs where humans and livestock are linked in many ways including direct contact due to close proximity, and shared environments receiving human and animal waste. The links between human and livestock populations provide an opportunity for either population to act as a reservoir from which AMR bacteria and/or their AMR determinants could be transmitted in either direction (Woolhouse et al., 2015; Robinson et al., 2016a). Livestock have been implicated as a reservoir for AMR bacteria and AMR genes that may spread to humans, with the keeping of livestock posited as a risk factor for AMR in humans. However, the relative contribution of livestock to AMR carriage among humans remains to be elucidated (Muloi et al., 2018).

Previous understanding of transmission of AMR bacteria and/or AMR determinants relied on low resolution typing tools and most studies have been limited by opportunistic sampling with little spatiotemporal overlap between humans and livestock. High-resolution analysis of bacterial genomes obtained from co-habiting humans and livestock using phylogenetic and ecological methods can help to shed light on important questions on direction and frequency of AMR transmission between humans and livestock. In this thesis, I used the ubiquitous *E. coli* as a marker to investigate the role of livestock keeping as a potentially high-risk urban interface for AMR transmission between humans and livestock in Nairobi, Kenya. The main hypothesis was that there is an epidemiologically significant spillover of AMR bacteria and AMR determinants from livestock to human populations.

In the following paragraphs, I will provide a summary of the key results of this thesis and discuss how they contribute to answering the main questions of this thesis. Further, I will discuss how such findings relate with current knowledge of AMR transmission and the broader implications of these results to epidemiology, animal, public and ecosystem health. Lastly, I will highlight the questions raised by this thesis and provide suggestions for the direction of future work before drawing the thesis to a conclusion.

9.1 Review of evidence supporting/refuting transmission of AMR bacteria from livestock to humans

In chapter three, I analysed the available evidence on the role of livestock as a source of resistant *E. coli* and their AMR determinants in humans and systematically reviewed the kinds of evidence used to support, or refute, the transfer of AMR bacteria to humans. I demonstrated that whilst eight (18%, n=45) studies describe transmission of AMR from livestock to humans, robust conclusions on the directionality of transmission are limited by study methodologies. The eight studies suggesting to provide evidence of directional transmission of AMR were broadly similar to 25 studies that suggested only overlap of resistance between the two populations. This finding provide evidence that demonstration of similarity of AMR bacteria or AMR determinants does not, by itself, provide evidence of directionality of transfer. Further, this analysis demonstrated that current understanding of AMR transmission is complicated by that fact that AMR studies have used different approaches, varying in the (i) resolution of phenotypic/molecular methods used to characterise strains, (ii) antimicrobials tested, and (iii) sampling frames and data collection approaches. In the discussion section, I highlighted major recommendations for future studies investigating the direction and frequency of AMR between human and livestock populations. Importantly, and in line with the rest of my thesis, I recommended the use of high resolution genomics methods on human and livestock bacterial

isolates collected in time and space in an epidemiologically-structured framework.

9.2 Antimicrobial resistance carriage at a community level

In chapters 4 and 5, utilising AMR phenotypes and genotypes respectively, I explored the variation in carriage of AMR *E. coli* between human and livestock populations, and investigated the role of livestock ownership as a risk factor for AMR carriage in humans. Chapter 4 focused on the epidemiology of clinically relevant AMR phenotypes in humans and urban livestock. 633 livestock and 321 human *E. coli* isolates were tested for susceptibility to 13 antimicrobial drugs representing 9 antimicrobial classes. Prevalence of resistance to sulfonamides, trimethoprim, tetracyclines, and aminoglycoside was consistently higher (>40% of resistant isolates) than that to other tested antimicrobials across both human and livestock isolates, and 47.6% and 21.1% of isolates displayed resistance to ≥ 3 and ≥5 antimicrobial classes respectively. Human, poultry and pigs had the highest prevalence of AMR *E. coli* carriage compared to goats, rabbits and cattle. At household interfaces, exchange of AMR between humans was influenced by human density, and the presence of livestock manure, but not livestock keeping by itself. I found evidence of a co-occurring phenotype (conferring resistance to tetracycline, sulfonamide and trimethoprim antimicrobial classes) common in both human and livestock isolates, suggestive of a conjugative plasmid disseminating MDR within the *E. coli* population in the host groups.

In chapter 5, I genomically characterized the carriage, diversity and assemblages of AMR genes within commensal *E. coli* populations from humans and livestock. By screening AMR genes, I detected 60 different acquired genes and 14 point mutations associated with reduced antimicrobial susceptibility present in variable proportions in human and livestock isolates. *sul2, strA, strB, tetA,* and $bla_{TEM\text{-}1B}$ AMR genes, conferring resistance to sulphonamides, aminoglycosides, tetracyclines and β-lactams respectively, were the most common AMR genes

across both host groups. Most (64/74, 86.5%) of the genes were found in similar proportions in both humans and livestock while the remainder (10/74, 13.5%) were significantly more common in human than in livestock isolates. Highest AMR gene carriage was observed in humans, pigs and poultry compared to rabbits, goats and bovines. However, AMR gene assemblages did not differ by host type or household location. Analysing the potential drivers of variation in AMR gene carriage in humans, I demonstrate that presence of livestock in the household did not influence AMR gene carriage in humans, but human AMR gene carriage was instead influenced by presence of animal manure in the household.

9.3 Tracking bacterial sharing in co-habiting humans and livestock

In chapter 6, I used high resolution genetic data to characterise *E. coli* populations, and to elucidate patterns of strain sharing as a proxy for transmission potential. Phylogenetic analysis of core genome demonstrated that livestock and patient isolates were genetically heterogeneous, with minimal evidence of clustering by host group, and as such my results suggest that *E. coli* is circulated widely between human and livestock populations. Genomic comparison of isolates revealed 91 sharing events differing by less than ten base pairs (59 involving livestock isolates only (mostly in poultry), 23 human isolates only, and 9 between humans and livestock). As a likely point of contact, hence an opportunity for bacterial transmission, I found evidence that, most of the sharing events were confined within households with instances of spread between households. By comparing the population structure of the human *E. coli* genomes with respect to livestock keeping status this thesis demonstrated that *E. coli* core genomes in humans did not segregate according to livestock ownership. Further, I demonstrated that high-resolution sequence-based analysis of SNPs is more discriminatory than MLST in typing of bacteria, in particular between commensal strains which have a high genetic diversity.

9.4 Antimicrobial usage patterns

Chapter 7 describes a survey I undertook to investigate the patterns of antimicrobials available for sale in urban Nairobi, as a proxy for antimicrobial usage in human and livestock populations. I found evidence of overlap in antimicrobial classes, including of critically important antimicrobials, available for use in human and veterinary medicine, with noticeable variations in the sale of some antimicrobial classes. Broad spectrum β-lactams, fluoroquinolones, first and second generation cephalosporins, and metronidazole were the most commonly purchased human antimicrobials while tetracyclines, sulphonamides, penicillins, and macrolides were the most commonly purchased veterinary antimicrobials. Colistin – a drug considered of last resort in human medicine – was an antimicrobial of choice amongst poultry farmers in 16% of veterinary drug stores. Analysing the level of awareness and common behaviours related to antimicrobial prescribing amongst human and veterinary pharmacists, I found that whilst most pharmacists showed high knowledge about antimicrobial use and AMR, inappropriate prescribing practices were common. In addition, over the counter sale of antimicrobials, without a prescription, was a common occurrence in both human veterinary drug stores.

9.5 Co-occurrence patterns of antimicrobial resistance genes

In chapter 8, I analysed the patterns of AMR gene co-occurrence and the distribution of conjugative plasmids in *E. coli* isolates from humans and livestock. I found evidence of a co-occurring AMR gene cluster comprised of *strA, strB*, and *sul2* genes frequently co-located with *tetA*, $bla_{TEM\text{-}1B}$ and *dfrA1* genes conferring resistance to aminoglycoside, sulfonamide, tetracycline, β-lactams and trimethoprim antimicrobial classes respectively. This co-location of multiple AMR genes, often on dispersive elements such as plasmids and transposons, enables acquisition and dissemination of multi-drug resistance phenotypes in a single step. I demonstrate evidence that two previously described plasmids (pCERC1 and pCERC2) were significantly associated with the co-located AMR

genes. Screening of plasmid replicons, I found 31 plasmid replicons distributed in similar proportions in human and livestock isolates, with the exception of colBS512 and IncB/O/K/Z significantly more common in humans, and p0111 and IncHI1B plasmids more common in livestock. Plasmid replicon carriage per isolate did not differ between human and livestock populations. I found evidence that AMR gene distribution was significantly correlated with the plasmid replicon composition, in particular, the ColRNAI, IncQ1 and p0111 plasmid backbones were significantly associated with carriage of the *strA, strB, sul2* AMR gene cluster.

9.6 Implications of this thesis

Results of this thesis have implication on the epidemiology of bacteria and AMR transmission between human and livestock populations and practical relevance to public and animal health. In the following section, I will discuss the how the results presented in this thesis fits with current literature and how the conclusions and limitations of the thesis highlight important areas for future work.

9.6.1 Expanded genomic approaches to further understand bacterial transmission

Previous studies have investigated the transmission epidemiology of AMR *E. coli* between human and livestock, but very few use high-resolution genetic data and samples col-located in time and space (Muloi et al., 2018). A smaller number have focused on developing countries, with none considering the role of the ubiquitous urban livestock in the maintenance of zoonotic bacteria and AMR in humans. By utilizing tools with the finest resolution available to investigate the associations of *E. coli* and AMR between and within co-located human and urban livestock populations, the approach used in this thesis is novel, and has enabled me to address some important epidemiological questions. First, the findings presented in chapter 4 and 5 demonstrate overlapping patterns of AMR phenotype and gene carriage in human and livestock populations, and most importantly, that livestock ownership, in and of itself, does not add to the risk of

acquisition or carriage of AMR bacteria in humans. Further, supporting this, findings in chapter 6 demonstrate that *E. coli* strains are largely shared within livestock and human populations separately with limited transmission in either direction and that human *E. coli* population is not differentiated by livestock ownership. The finding that most bacterial sharing was limited within the same host group may partly be attributed to the fact that for most livestock species, as well as most humans, most of direct contact is confined within members of the same species. Taken together, these findings relate directly to the findings in (Muloi et al., 2018), and support a small, but growing, body of evidence suggesting that livestock plays a minimal role in acquisition and infection of AMR bacteria in humans (Mather et al., 2013; Gouliouris et al., 2018; Ludden et al., 2019a; Ludden et al., 2019b).

Second, understanding how pathogen communities are structured is a fundamental step towards developing a predictive framework for pathogen emergence at urban livestock-human interfaces including AMR bacteria. In this study, the dispersal of bacteria and AMR determinants (AMR phenotypes, AMR genes and plasmids) and lack of structure in microbial community by host or wealth categories exemplifies the Baas Becking tenet 'everything is everywhere, but the environment selects' (Becking, 1934), suggesting that the hypothesis also applies to genes, besides microbes for which it was originally formulated. 'Everything is everywhere' alludes to the dispersal potential of *E. coli*, whereas 'the environment selects' denotes the specifically adapted bacterial clones that have the ability to thrive and proliferate in a particular environment, for example the closely related lineage of goat *E. coli* isolates observed in chapter 6. By doing so, these analyses demonstrates that, across the fragmented urban landscapes, AMR genes act as 'public goods' and are available for bacteria in different ecological niches to integrate into their genomes. Besides describing the pathways for the distribution of bacteria and AMR gene pools, this analysis

provides important biological insights into the dispersal of AMR determinants across multiple hosts and habitats.

Finally, this study highlights the added value of integrated epidemiological and genomic approaches in tracking transmission of AMR bacterial clones and in surveillance of AMR genetic determinants in complex multi-host-pathogen interfaces (Baker et al., 2018b). Analysis of fine-scale genetic variation in this thesis has demonstrated evidence of bacterial sharing within and between host groups and revealed evidence that commensal *E. coli* population is diverse. Further, this thesis exploits WGS to investigate the epidemiology of AMR genes and plasmids – important genomic resistance markers. Given the epidemiological significance of horizontal genetic transfer mechanisms in rapid dissemination of AMR genes (Sheppard et al., 2016; Baker et al., 2018a) detailed genetic analysis in this thesis highlights the added value of focusing on these dispersive elements when tracking transmission of AMR.

9.6.2 Implications for surveillance and public health

The widespread carriage of clinically relevant AMR phenotypes and AMR genes in both human isolates reported in this thesis support previous findings (Kalter et al., 2010; Nguyen et al., 2015; Caudell et al., 2018; Ingle et al., 2018; Williams et al., 2018) that AMR is increasingly common across LMICs. This thesis provides data regarding AMR frequencies for a range of antimicrobials in human and livestock populations – a significant contribution to the information gap of AMR in LMICs. My results reiterate the need for routine surveillance activities of AMR in low income settings and for public health policy to adopt effective strategies targeting a reduction in the emergence and spread of such resistance in the future. Global platforms such as World Health Organization's Global AMR Surveillance System (GLASS) could offer a cost-effective model to conduct surveillance for AMR bacteria in LMICs (Seale et al., 2017), and could be integrated with existing systems for reporting of zoonoses in human and animal

health. The cost-effectiveness of the surveillance programs could be enhanced by focusing on particular resistance phenotypic or genetic markers deemed of more clinical relevance (DANMAP, 2018). The use of WGS in this thesis in a low income settings is important given the concerted effort to implement WGS in public health settings as the cost of sequencing declines. In clinical and public health settings, genomics would aid the characterising and tracking of bacterial spread and provide critical surveillance data on genomic mechanisms of resistance.

Phenotypic resistance profiles and genotypes detected in the *E. coli* isolates (chapter 4 and 5) correlated with the most commonly used antimicrobial in the study (chapter 7). Further, over the counter dispensing of non-prescribed human and veterinary antimicrobials was common across Nairobi (chapter 7). Antimicrobial use is singlehandedly the most important driver of AMR in both humans and livestock (Holmes et al., 2016) and recent studies suggest that antimicrobial consumption rates in LMICs have surpassed those observed in high income countries (Klein et al., 2018). As the urban population in most low and middle-income countries grows (UNPD, 2014b), disease burden and consequent increase in antimicrobial usage is projected to rise (Sosa et al., 2010). As such, findings of this research could be adopted into policy recommendations aimed at selectively reducing inappropriate use of antimicrobials, increasing appropriate use of antimicrobials to treat and prevent disease, and reducing the need for antimicrobials (Laxminarayan and Heymann, 2012). Programmes on rational antimicrobial use or antimicrobial stewardship in the community and the clinic should be initiated. While this thesis reports high AMR carriage in the community, public health policy should balance the need to reduce overall antimicrobial use with expanding essential access as lack of access to antimicrobial still results in more deaths in low income settings than does AMR (Das and Horton, 2016).

To reduce the carriage of AMR in humans across household interfaces this thesis highlights some interventions that could be implemented at local and policy

level. For example, in chapter 4 and 5, disposal of animal manure in the household was identified as a risk factor for carriage of AMR in humans. Strategies that limit AMR gene flow to and from manure (to humans) could be adopted. Such interventions include educating people on safe disposal of manure from households, and manure pre-treatment prior to application onto crop farms where possible.

9.7 Future directions

In chapter 6, I demonstrate evidence of bacterial sharing within and between human and livestock population, in either direction. Directionality of bacterial and AMR sharing between human and livestock populations has been subject of much debate with recent studies suggesting that some MRSA clones infecting humans may have originated from livestock (Lowder et al., 2009; Ward et al., 2014; Richardson et al., 2018). This thesis does not provide insights on the direction of bacterial and/or AMR transmission as datasets used here were cross sectional snapshots, but the use of longitudinal sampling of human and animal populations (over time and space) combined with phylogeographic inferences (De Maio et al., 2015) will provide robust insights into transmission history in the near future.

In chapter 4, I used disc diffusion for antimicrobial susceptibility testing and Clinical and Laboratory Standards Institute (CLSI, 2016) guidelines for interpretation of inhibition zone diameters. Breakpoint setting and use has, and still is, a controversial subject and the focus of much debate in microbiology and clinical infectious disease research (MacGowan and Wise, 2001; Turnidge and Paterson, 2007). Evidently, the pre-established clinical cut offs did not accurately reflect the distribution of inhibition zone diameters in the bacterial population in this study. Despite this limitation, breakpoints are an integral part of modern microbiology laboratory practice globally, and most importantly in low resource settings such as Nairobi. In the recent past, a small number of studies have assessed the feasibility of using WGS to predict antimicrobial susceptibility

phenotype (genotype-to-phenotype), in *Staphylococcus aureus* (Gordon et al., 2014), *E. coli* (Stoesser et al., 2013; Ingle et al., 2018) and *Mycobacterium tuberculosis* (Bradley et al., 2015). However, none of these studies have focused on commensal bacteria or isolates of livestock origin. Such research questions represent the next step towards understating whether genetic predictions could also be used to guide clinical decision making and for surveillance purposes.

In chapter 6 and 8, I hypothesised that the widespread carriage of acquired AMR and co-occurrences between clinically relevant resistance genes was mediated by conjugative plasmids that are shared between human and livestock populations. However, owing to shortcomings in reconstructing plasmids and other mobile elements encoding resistance genes from short read data, I was unable to determine the precise location of AMR genes and associated mobile genetic elements. It would be beneficial for additional studies to characterise plasmids using long-read sequencing to accurately reconstruct all plasmids in order to understand mechanisms by which AMR determinants may be acquired and dispersed (Conlan et al., 2014; Rozwandowicz et al., 2018).

By only sequencing a single *E. coli* isolate from each host, this study may have underestimated both within-host strain and AMR mechanism diversity and potential sharing of strains between hosts (Stoesser et al., 2015). The decision to sequence a single isolates from each host was made as a cost-based trade-off between the depth of sampling *E. coli* genetic diversity within each individual and the number of unique individuals from which samples could be included. Recent advances in metagenomics permit sequencing of bacterial microbiomes, which, when used in combination with structured epidemiological data could be used to explore the variation in the structure of bacterial and AMR communities within and between human and livestock populations (Pehrsson et al., 2016; Hendriksen et al., 2019).

Chapter 7 used a relatively small sample size (19 and 40 veterinary and human drug stores respectively) to analyse antimicrobial sales in human and veterinary drug stores in Nairobi, as proxy for antimicrobial usage in animal and human populations. By doing do this, this thesis may not have reliably captured the real antimicrobial usage patterns in either population. Also, considering the many sources of sales of antimicrobials across the city, including informal sources, it is possible that I missed some of these during data collection hence underestimating use. It would be interesting for future studies to extend this work to longitudinal surveys of antimicrobial use in healthcare facilities and the community.

9.8 Concluding remarks

The emergence and transmission of AMR bacteria poses an immediate threat to global health, and the role of livestock in transmission of AMR bacteria to humans is unclear. This thesis has demonstrated how fine-scale analysis of bacterial genomes explicitly embedded within an epidemiologically structured sampling framework can be utilized; (i) in the surveillance of AMR prevalence, (ii) to identify risk factors for carriage of AMR strains and AMR genes, (iii) to track bacterial strain sharing in a low-income urban setting. I provide evidence that human and livestock bacterial and AMR communities overlap, but livestock ownership in and of itself does not add to the risk of acquisition or carriage of AMR bacteria in humans. At the SNP level, bacterial sharing is largely confined within livestock and human populations with limited evidence of transmission in either direction. The work presented herein offers insights into the epidemiology of AMR in commensal bacteria in humans and their livestock in a low income developing city and reaffirms the need for enhanced surveillance of resistant bacteria as well as antimicrobial sales and usage in both populations.

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Chapter 10

Bibliography

> If I have seen further, it is by standing on the shoulders of Giants
>
> **Isaac Newton, 1676**

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Chapter 10

Appendices

> “Messieurs, c'est les microbes qui auront le dernier mot."
> Gentlemen, it is the microbes who will have the last word.
> **Louis Pasteur, 1822-1895**

Chapter 11 Appendices

11.1 Appendix A. Are Food Animals Responsible for Transfer of Antimicrobial-Resistant *Escherichia coli* or Their Resistance Determinants to Human Populations? A Systematic Review

Appendix Table A1. Summary of 45 included studies

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
No matching	Active	Poultry, 119	Clinical, 100 Healthy, 117			β-lactams, Chloramphenicol, Gentamicin, Spectinomycin, Tetracycline, Nitrofurantoin, Trimethoprim/sulphamethoxazole, Ceftazidime	AST and Serotyping	Animal to human	Low resolution typing	Clonal	Overlapping serotypes High spectinomycin resistance in humans and poultry isolates despite limited use in humans
Ueda et al., 2015	Vietnam, 2013	Poultry,47	Healthy, 199	Spatial and temporal	Active	Cephalosporins	AST, PCR for gene detection, MLST for genotypic relatedness and replicon typing for plasmid characterisation	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal, ARGs and plasmid profiles
Jakobsen et al., 2015	Denmark, 2006-2010	Cattle, 4 Poultry,3 Pigs, 21 Horses,15	Clinical,22	Spatial and temporal	Active	Cephalosporins	PFGE for genotypic relatedness and PCR-based replicon typing and pMLST and restriction fragment length polymorphism (RFLP) for plasmid characterisation	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal and plasmid profiles
Tseng et al., 2015	Taiwan, 2013	Pigs, 22	Clinical,123	Spatial and temporal	Active	Cephalosporins, Fosfomycin	AST, PFGE and MLST for genotypic isolate relatedness, PCR for gene characterisation and plasmid characterisation	Animal - human overlap	Intermediate resolution typing	Determinant	Distinct clonal profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Lupindu et al., 2015	Tanzania, 2012	Cattle, 50 Soil, 21 Water,7	Healthy, 40	Spatial and temporal	Active	β-lactams, Tetracycline	AST and PFGE for isolate genotypic relatedness	Animal - human overlap	Low resolution typing	Clonal	Overlapping clonal profiles
Huijbers et al., 2015	Netherlands, 2011-2012	Poultry,70	Healthy, 27	Spatial and temporal	Active	Cephalosporins	AST, MLST for isolate genotypic typing and PCR for gene characterisation	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping ARG profiles
Valentin et al., 2014	Germany, 2011-2014	Cattle,120 Poultry,49 Pigs,139	Clinical,422 Healthy,213	No matching	Active	Cephalosporins	AST, PCR for gene and isolate genotypic characterisation	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal and ARG profiles
Hammerum et al., 2014	Denmark, 2010-2011	Pigs, 339	Healthy, 195	Spatial and temporal	Active	Cephalosporins	AST,PCR for gene detection, PFGE and MLST for isolate genotypic typing, and PBRT for plasmid replicon typing	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal and ARG profiles
Hu et al., 2013	China, 2012-2013 & archived isolates	Pigs, 31 Water, 26	Clinical, 36 Healthy, 46	No matching	Active & Passive	Cephalosporins	AST,PCR for gene detection, PFGE and MLST for isolate genotypic typing	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal and ARG profiles
Ciccozzi et al., 2013	Italy, 2009-2010	Poultry, 43	Clinical, 129	No matching	Passive	β-lactams, Trimethoprim/sulfamethoxazole, Gentamicin, Cephalosporins Ciprofloxacin	AST, phylogenetic analysis of MLST sequence type sequences	Animal - human overlap	Intermediate resolution typing	Clonal	Overlapping clonal profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Dierikx et al., 2013	Netherlands, 2009	Poultry, 26	Healthy, 18	Temporal	Active	Cephalosporins	AST, Microarray analysis and PCR for gene characterisation, MLST for isolate genotypic typing and RFLP for plasmid typing	Animal to human	Intermediate resolution typing	Determinant	High prevalence of resistance in poultry isolates compared to human isolates Overlapping ARG and plasmid profiles
Stokes et al., 2012	United Kingdom, 2006-2010	Cattle, 33 Poultry, 9 Sheep, 2	Clinical, 26	No matching	Passive	Cephalosporins	PFGE and DNA arrays for isolate typing, and RFLP for plasmid characterisation	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping plasmid profiles
Johnson et al., 2012	USA, Netherlands and Japan, 1990-2005	Broilers and Poultry, 1331	Patients, 629 Healthy, 252	No matching	Passive	Sulfonamides, Tetracyclines, Macrolides, β-lactams, Chloramphenicol, Ciprofloxacin, Gentamicin, Quinolones,	AST, PCR for ARGs and virulence genes characterisation, and PRBT for plasmid typing	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal, ARG and plasmid profiles
Deng et al., 2011	China, 2002	Pigs, 24 Environment, 5	Healthy, 12	Spatial and temporal	Passive	Fluoroquinolones	AST,PCR for ARGs characterisation, PFGE and MLST for isolate genotypic typing, and PBRT for plasmid replicon typing	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal, ARG and plasmid profiles
Zhao et al., 2010	China, 2002	Poultry, 25 Pigs, 73	Healthy, 33	Spatial and temporal	Active	Quinolones, Ciprofloxacin	AST,PCR for ARGs characterisation, PFGE and MLST for isolate genotypic typing, and RLFP for plasmid typing	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal and ARG profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Schwaiger et al., 2010	Germany, 2002-2004	Pigs, 137	Patients,152	No matching	Active	Tetracyclines, Sulfonamides, Streptomycin	AST,PCR for ARGs characterisation	No transfer	Intermediate resolution typing	Determinant	Distinct ARG profiles
Moodley & Guardabassi, 2009	Denmark, 2007	Pigs, 70 Environment, 35	Healthy, 5	Spatial and temporal	Active	Cephalosporins	AST,PCR for ARGs characterisation, PFGE for isolate genotypic typing and, RLFP for plasmid typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping plasmid profiles
Mulvey et al., 2009	Canada, 1999-2000	Cattle,51	Patients, 25	Temporal	Passive	Cephalosporins	AST,PCR for ARGs characterisation, PFGE for isolate genotypic typing and plasmid typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping plasmid profiles
Ho et al., 2009	China, 2002-2004	Cattle, 11 Poultry, 25 Pigs, 28	Patients, 51 Healthy, 59	No matching	Passive	Trimethoprim-Sulfamethoxazole	AST,PCR for ARGs and integron characterisation, and PFGE for isolate genotypic typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping integron and ARG profiles
Graziani et al., 2009	Italy, 2006	Poultry, 113	Healthy, 125	No matching	Passive	Ciprofloxacin	AST,PCR and PFGE for isolate genotypic and virulence gene typing	No transfer	Intermediate resolution typing	Clonal	Distinct clonal profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Hammerum et al., 2006	Denmark, 2002-2003	Pigs, 610 Pork, 189	Healthy, 199	No matching	Active	Sulfonamides	AST,PCR for ARGs characterisation	Animal to human	Intermediate resolution typing	Determinant	Overlapping ARGs in animal and human isolates High prevalence of resistance in animal isolates compared to human isolates
Johnson et al., 2006	Spain, 1996-1998	Poultry, 49	Healthy, 68	Spatial and temporal	Passive	Ciprofloxacin	AST,PCR and PFGE for genotypic and virulence gene typing	Animal to human	Intermediate resolution typing	Clonal	High similarity in resistant animal and human isolates
Maynard et al., 2004	Canada, 2001	Pigs, poultry, cattle and pets, 39	Patients, 70	No matching	Active	β-lactams, Aminoglycosides, Tetracycline, Phenicols, Trimethoprim, Sulfonamides	AST,PCR and colony hybridisation for ARGs characterisation and distribution	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal profiles
Kang et al., 2005	South Korea, 2001-2003	Poultry, 163 Pigs, 133	Patients,201 Healthy,167	Temporal	Active	Trimethoprim, Aminoglycosides, Sulfonamides	AST,PCR for integron characterisation, PFGE for genotypic typing, and plasmid typing	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal and plasmid profiles
Oppegaard et al., 2001	Norway, 1996	Cattle, 13	Healthy , 7	Spatial and temporal	Active	β-lactams, Tetracycline, Sulfonamides, Streptomycin, Trimethoprim	AST,PCR for integron characterisation, serotyping, and plasmid typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping plasmid profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Bogaard et al., 2001	Netherlands, 2001	Poultry, 122	Healthy, 216	Spatial and temporal	Active	Ciprofloxacin	AST, PFGE for isolate genotypic typing	Animal to human	Low resolution typing	Clonal	High prevalence of resistance in animal isolates compared to human isolates Overlapping clonal profiles
Kariuki et al., 1999	Kenya, 1994	Poultry, 248	Healthy,128	Spatial and temporal	Active	β-lactams, Aminoglycosides, Tetracycline, Chloramphenicol, Trimethoprim, Ciprofloxacin, Quinolones,	AST, PFGE for isolate genotypic typing, and plasmid typing	No transfer	Low resolution typing	Clonal	Distinct clonal profiles
Leverstein-van Hall et al., 2011	Netherlands, 2006-2010	Poultry, 35 Poultry meat, 81	Patients,409	No matching	Passive	Cephalosporins	AST, microarray analysis for ARGs characterisation, MLST for isolate genotypic typing, and PBRT for plasmid typing	Animal to human	Intermediate resolution typing	Clonal and determinant	Overlapping clonal, ARG and plasmid profiles
Jakobsen et al., 2011	Denmark, 2004	Poultry, 17 Pigs, 8 Pork, 30 Broiler meat,18	Patients, 52 Healthy, 36	Spatial and temporal	Active	β-lactams, Chloramphenicol, Trimethoprim, Ciprofloxacin, Quinolones, Macrolides	Microarray analysis for ARGs and virulence genes characterisation	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal and ARG profiles
Vieira et al., 2011	Europe, 2005-2008	Poultry, 4000 Pigs, 4500 Cattle, 3500	Patients, 100,000	Temporal	Passive	β-lactams, Ciprofloxacin, Aminoglycosides, Cephalosporins	AST	Animal - human overlap	Co-occurrence	Clonal	Significant correlations between human and animal isolates

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Ho et al., 2010	China, 2002-2004	Cattle, 11 Poultry, 37 Pigs, 33 Fish, 1	Patients, 167	No matching	Active	Aminoglycosides	AST, PCR for ARGs characterisation, PCR and PFGE for isolate genotypic typing, and PBRT for plasmid typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping ARG and plasmid profiles
Xia et al., 2010	China, 2007	Poultry,198 Pigs, 137	Patients,139 Healthy,18	Spatial and temporal	Active	Fluoroquinolones	AST, PCR for ARGs characterisation, PCR and PFGE for isolate genotypic typing	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal patterns
Dohmen et al., 2015	Netherlands, 2011	Pigs, 160	Healthy, 13	Spatial and temporal	Active	Cephalosporins	AST, PCR for ARGs characterisation, PCR and MLST for isolate genotypic typing, and PBRT for plasmid typing	Animal - human overlap	Intermediate resolution typing	Clonal and determinant	Overlapping clonal, ARG and plasmid profiles
Kariuki et al., 1997	Kenya, 1993-1994	Poultry, 246	Patients,168	Spatial and temporal	Active	β-lactams, Tetracycline, Chloramphenicol, Cephalosporins, Ciprofloxacin, Quinolones,	AST and restriction endonuclease digestion (RED) for plasmid typing	No transfer	Low resolution typing	Determinant	Distinct clonal patterns
Phongpaichit et al., 2007	Thailand, 2004-2005	Pigs, 432	Healthy, 185	Spatial and temporal	Active	β-lactams, Aminoglycosides, Tetracycline, Chloramphenicol, Cephalosporins, Trimethoprim-Sulfamethoxazole, Ciprofloxacin, Quinolones, Macrolides	AST and PCR for ARGs and integron characterisation	No transfer	Intermediate resolution typing	Determinant	Distinct integron patterns

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Riccobono et al., 2012	Bolivia, 2002-2005	Poultry, 36	Healthy, 30	Spatial and temporal	Active	Tetracyclines, Quinolones	AST and PCR for ARGs characterisation and genotypic typing	No transfer	Intermediate resolution typing	Clonal and determinant	Distinct clonal and plasmid profiles
Zhang et al., 2009	China, 2004-2007	Poultry, 106 Pigs, 172	Healthy, 23	Spatial and temporal	Active	Aminoglycosides, Trimethoprim, Sulfonamides	AST and PCR for ARGs and integron characterisation	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping ARG and integron profiles
Levy, 1978	USA 1974	Poultry, 320	Healthy, 337	Spatial and temporal	Active	Tetracyclines	AST	Animal to human	Low resolution typing	Clonal and determinant	Overlapping clonal profiles
de Been et al., 2014	Netherlands, 2006-2011	Poultry, 4 Poultry meat, 7 Pig, 4	Healthy, patients, 17	No matching	Passive	Cephalosporins	AST, MLST and WGS for genotypic typing, and phylogenetics for relatedness analysis	Animal - human overlap	High resolution typing	Determinant	Increase in antimicrobial-resistance in humans in contact with both tetracycline-fed chickens and oxytetracycline supplemented feed
Jorgensen, 1983	Denmark, Britain 1960-1981	Pigs, 19	Patients, 13	No matching	Passive	Cephalosporins	AST and hybridisation techniques for plasmid analysis	Animal - human overlap	Low resolution typing	Clonal and determinant	Overlapping plasmid profiles
Dahms et al., 2015	Germany, 2012	Cattle, 10 Pig, 33 Poultry, 13	Healthy, 5	Spatial and temporal	Active	B-lactam, Chloramphenicol, Ciprofloxacin, Streptomycin, Sulfamethoxazole, Tetracycline, Trimethoprim	AST, PCR for ARGs characterisation, MLST for isolate genotypic typing	Animal - human overlap	Intermediate resolution typing	Clonal	Overlapping clonal and ARG profiles

Author, Year of publication	Sample origin and collection date	Source and No. of animal isolates	Source and No. of human isolates	Matching	Study type	Antimicrobials reported	Methods used	Direction stated	Nature of evidence	Nature of transfer	Evidence for nature of transfer
Jakobsen et al., 2010	Denmark, 2004-2006	Broiler meat, 283 Pork, 187 Poultry, 138 Pigs, 145	Patients, 102 Healthy, 109	Spatial and temporal	Active	Ciprofloxacin	AST, PCR for genotypic typing	Animal to human	Intermediate resolution typing	Clonal and determinant	Overlapping clonal profiles
Giufre et al., 2012	Italy, 2009	Poultry, 101	Patients, 277	Temporal	Passive	Chloramphenicol	AST, PCR for ARGs characterisation, PCR and MLST for isolate genotypic typing	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping clonal profiles
Winokur et al., 2001	USA, 1998-2000	Cattle &pigs, 377	Patients, 1017	Temporal	Active	β-lactams	AST , isoelectric focus analysis for ARGs characterisation, PFGE for genotypic typing and RFLP for plasmid analysis	Animal - human overlap	Intermediate resolution typing	Determinant	Overlapping plasmid and ARG profiles
Smet et al., 2009	Belgium, 2001-2007	Poultry, 4 Pigs, 2	Healthy, 3	No matching	Active	Cephalosporins	AST , PCR and isoelectric focus analysis for ARGs characterisation, and PBRT and RFLP for plasmid analysis	Animal - human overlap	Intermediate resolution typing	Determinant	Smet et al., 2009

Abbreviations: AST, antimicrobial susceptibility testing; PCR, polymerase chain reaction; ARGs, antimicrobial resistance genes; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; RFLP, restricted fragment length polymorphism; pMLST plasmid multilocus sequence typing; PBRT, PCR based replicon typing.

Appendix Table A2. Data search strategies

Search strategy for PubMed

((((((((((((antibiotic resistan*) OR antimicrobial resistan*) OR drug resistan*) AND Escherichia coli) OR E. coli) OR Enterobacteria*) AND human*) AND livestock) OR food animal*) OR pig*) OR swine) OR poultry) OR bovine*) OR cattle) OR cow*)

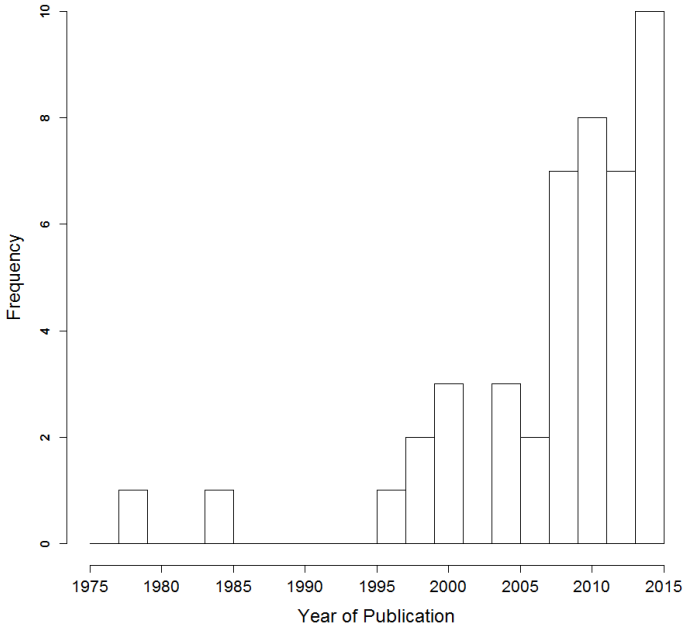
Search strategy for Web of Science and SCOPUS

(antibiotic resistan*) OR (antimicrobial resistan*) OR (drug resistan*) AND (Escherichia coli) OR (E. coli) OR (Enterobacteria*) AND (human*) AND (livestock) OR (food animal*) OR (pig*) OR (swine) OR (poultry) OR (bovine) OR (cattle) OR (cow*)

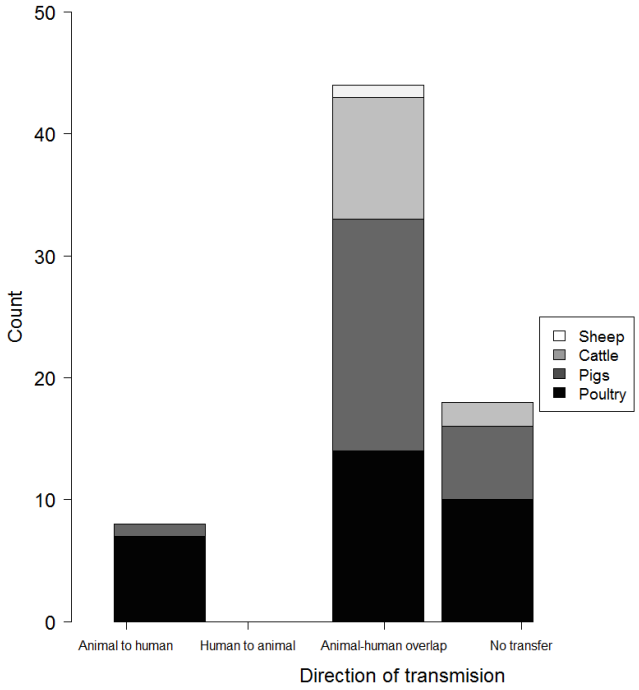
Search strategy for EMBASE

1. antibiotic resistance/exp
2. antibiotic resistance/ exp
3. drug resistance/ exp
4. Escherichia coli/ exp
5. Enterobacteria/ exp
6. human/ exp
7. livestock/ exp
8. food animal/ exp
9. pig/ exp
10. swine/ exp
11. poultry/ exp
12. bovine/ exp
13. cattle/ exp
14. cow/ exp
15 1 or 2 or 3
17. 4 or 5
18. 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
19. 6 and 15 and 17 and 18

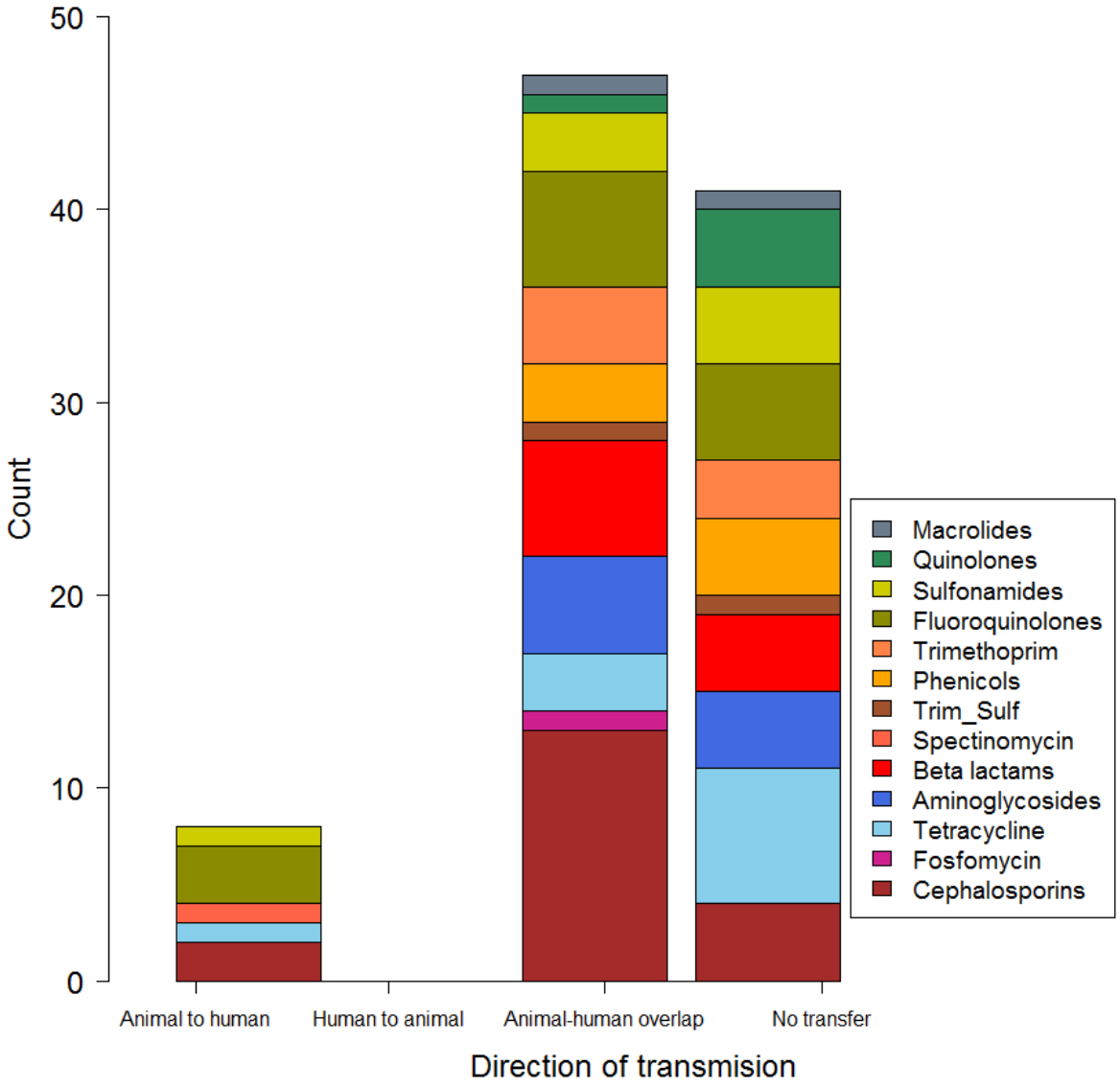
Appendix Figure A1. Annual frequency of studies in our review



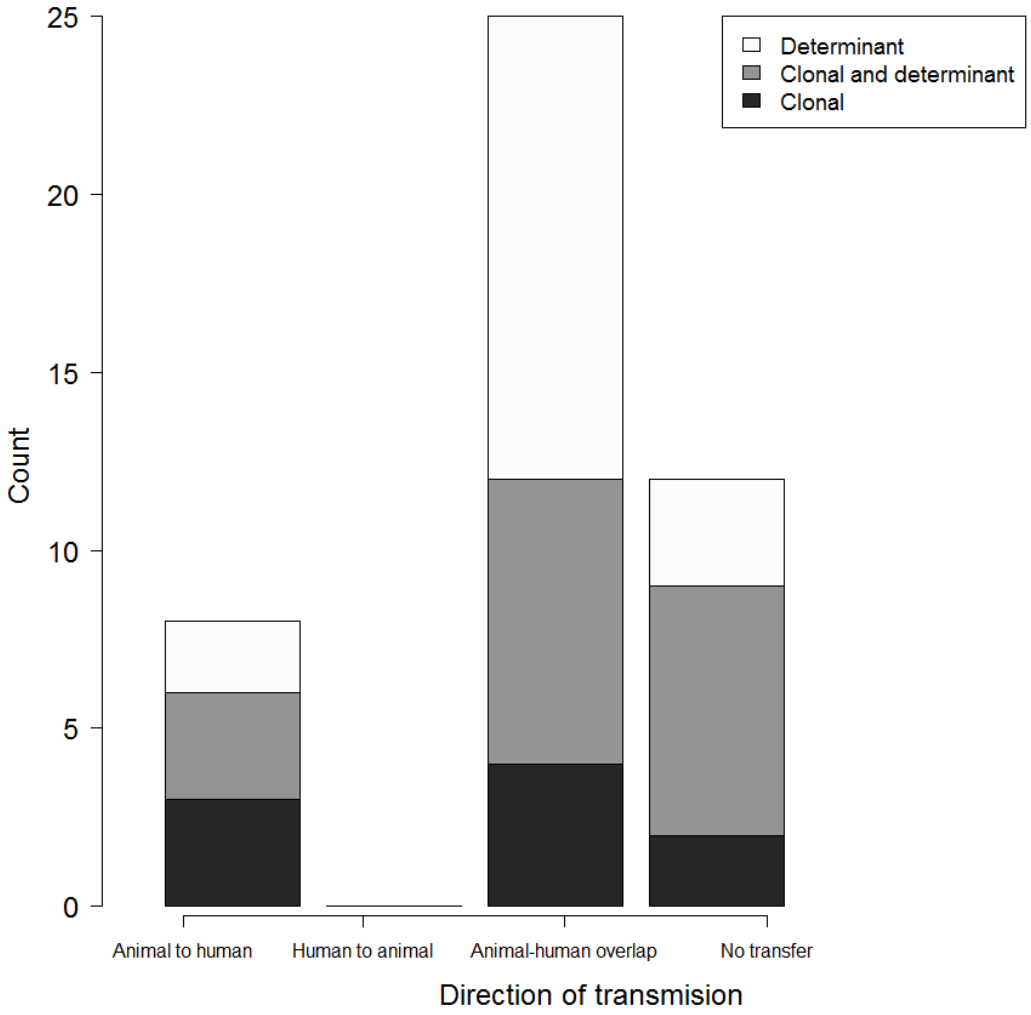
Appendix Figure A2. Counts of food animal species reported.



Appendix Figure A3. Counts of antimicrobials reported. Note that some studies reported more than one antimicrobial. (*Trim_Sulf =Trimethoprim/Sulfamethoxazole)



Appendix Figure A4. Nature of transfer



Appendix B for: Epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanising city

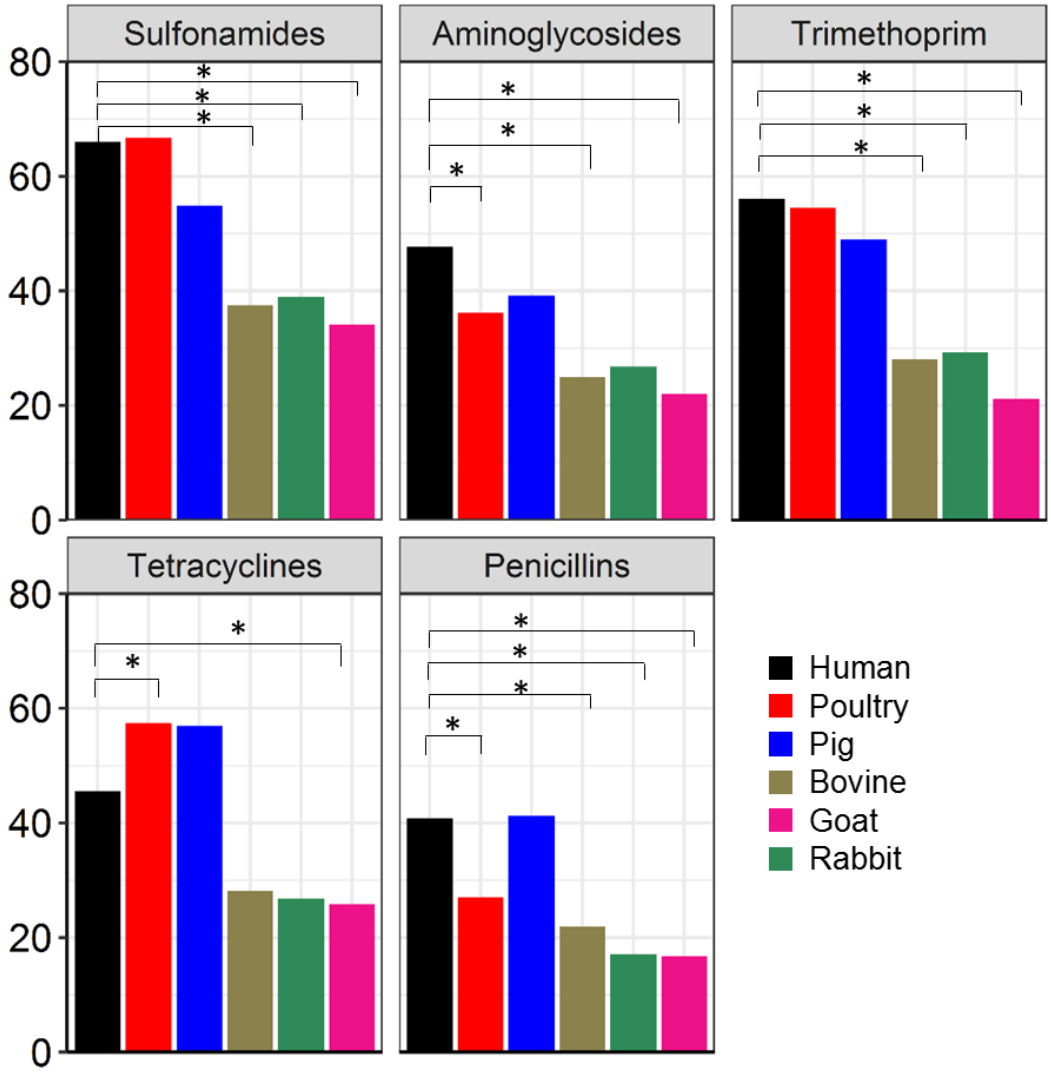
Appendix Table B1. Antimicrobials tested (and the respective antimicrobial classes) and interpretation of zone of inhibition (mm). * Custom breakpoints based on examination of the distributions of the zones of inhibition.

Antimicrobial class	Antimicrobial	Disc Content µg	Breakpoints Used (mm) Susceptible	Resistant
β-lactam antimicrobial	Amoxicillin-clavulanic acid	10	≥18	<13
Penicillin	Ampicillin	10	≥17	<13
Cephalosporin	Cefepime	30	≥25	<18
	Cefotaxime	30	≥26	<22
	Ceftazidime	30	≥21	<17
Fluoroquinolone	Ciprofloxacin	10	≥21	<15
	Nalidixic acid	30	≥19	<13
Aminoglycoside	Streptomycin	25	≥15	<11
	Gentamycin	10	≥15	<12
Phenicol	Chloramphenicol	30	≥18	<12
Sulfonamide	Sulfamethoxazole	30	≥17	<12
Folate pathway/acid inhibitor*	Trimethoprim	2.5	≥16	<10
Tetracycline*	Tetracycline	30	≥15	<11

Appendix Table B2. Percentages of *E. coli* isolates resistant to a panel of 13 antimicrobials classified by host type. Numbers show percentages of isolates classified as resistant based on the zone of inhibition. Definition of resistance is based on breakpoints used as described in the Material and Methods.

Antimicrobial class	Drug	Human (n=321)	Poultry (n=345)	Pig (n=51)	Bovine (n=64)	Goat (n=132)	Rabbit (n=41)
β-lactam	Co-amoxiclav	2.5	0.6	3.9	1.6	0	2.4
Penicillins	Ampicillin	40.8	27	41.2	21.9	16.7	17.1
Phenicols	Chloramphenicol	6.5	3.5	0	3.1	1.5	2.4
Cephalosporins	Ceftazidime	1.6	1.4	2	0	1.5	0
	Cefotaxime	2.2	4.3	2	4.7	3.8	0
	Cefepime	2.2	0.9	2	0	0.8	0
Fluoroquinolones	Ciprofloxacin	2.2	2.6	0	0	1.5	4.9
	Nalidixic acid	9.7	6.7	3.9	0	3	9.8
Sulfonamides	Sulfamethoxazole	66	66.7	54.9	37.5	34.1	39
Tetracyclines	Tetracycline	45.5	57.4	56.9	28.1	25.8	26.8
Aminoglycosides	Streptomycin	47	35.4	39.2	25	22	26.8
	Gentamicin	2.5	2.6	0	1.6	1.5	4.9
Trimethoprim	Trimethoprim	56.1	54.5	49	28.1	21.2	29.3

Appendix Figure B1. Percentages of *E. coli* isolates resistant to five antimicrobial classes by source type: human (n=321), poultry (n=345), pig (n=51), bovine (n=64), goat (n=132), and rabbit (n=41). P values are from a post-hoc Tukey's test. Only antimicrobial classes (5/9) with statistically significant differences in the percentages of resistance between the sources types are shown.



Appendix Table B3: Results of separate Poisson generalised linear mixed models investigating household risk factors for individual resistances by class in humans at the household level. Households not keeping livestock and having no manure in the household were used as the reference level in all models. Given the low prevalence of resistance (<5%) to co-amoxiclav, phenicols, cephalosporins and fluoroquinolones in our dataset, we did not include these in this analysis.

Variable	Estimate	Standard error	P value
Model 1: AMR carriage, humans in all households			
Tetracyclines			
Human density	0.6432	0.2069	0.00188
Large livestock (with or without small livestock)	0.1249	0.3068	0.68405
Small livestock only	0.4232	0.2849	0.13737
Aminoglycosides			
Human density	0.6329	0.2213	0.00424
Large livestock (with or without small livestock)	-0.1438	0.324	0.65711
Small livestock only	-0.2107	0.3011	0.48398
Sulfonamides			
Human density	0.61873	0.22858	0.00679
Large livestock (with or without small livestock)	0.07985	0.31112	0.79745
Small livestock only	0.55916	0.29782	0.06045
Penicillins			
Human density	0.7688	0.2304	0.000847
Large livestock (with or without small livestock)	-0.639	0.3371	0.058015
Small livestock only	-0.1738	0.3048	0.568543
Trimethoprim			
Human density	0.3728	0.2054	0.0695
Large livestock (with or without small livestock)	-0.2668	0.3013	0.3759
Small livestock only	0.1893	0.2819	0.5019
Model 2: AMR carriage, humans in livestock keeping household only			
Tetracyclines			
Human density	0.7179	0.2599	0.005733
Manure in household	0.3683	0.313	0.239321

Sulfonamides			
Human density	0.5369	0.2866	0.061
Manure in household	0.3933	0.3142	0.211
Aminoglycosides			
Human density	0.6915	0.2926	0.01812
Manure in household	0.1996	0.3507	0.56919
Penicillins			
Human density	0.6728	0.2571	0.00886
Manure in household	0.566	0.3384	0.09435
Trimethoprim			
Human density	0.2467	0.2508	0.325
Manure in household	0.456	0.3049	0.135

Appendix Table B4. Profile composition and number of *E. coli* isolates from human and animal in Nairobi, Kenya. Abbreviations: Cephalosporins (Ceph), Aminoglycosides (Amg), Phenicols (Phn), Tetracyclines (Tet), Penicillins (Pcn), Penicillin lactams (Amc), Folate inhibitors (Tmp), Sulfonamides (Sul) and Fluoroquinolones (Fq).

Profile composition	Human	Poultry	Bovine	Goat	Pig	Rabbit
Pansusceptible	67	76	33	72	17	19
Amc	0	0	1	0	1	0
AmcTmp	0	0	0	0	1	0
Amg	9	9	2	5	0	2
AmgAmc	1	0	0	0	0	0
AmgFqPhnSulTmp	0	2	0	0	0	0
AmgFqSul	0	0	0	0	0	1
AmgFqTetSulTmp	2	4	0	1	0	1
AmgPcn	0	1	2	0	0	1
AmgPcnAmcFqTetSulTmp	1	0	0	0	0	0
AmgPcnAmcTetSul	0	1	0	0	0	0
AmgPcnAmcTetSulTmp	2	1	0	0	0	0
AmgPcnFqPhnTetSulTmp	1	0	0	0	0	0
AmgPcnFqTet	1	0	0	0	0	0
AmgPcnFqTetSulTmp	9	6	0	1	1	0
AmgPcnPhnSulTmp	6	0	0	0	0	0
AmgPcnPhnTet	1	0	0	0	0	0
AmgPcnPhnTetSulTmp	6	1	2	0	0	0
AmgPcnSul	5	3	0	0	0	0
AmgPcnSulTmp	11	1	1	1	0	1
AmgPcnTet	2	4	0	1	1	0
AmgPcnTetSul	3	3	1	0	0	1
AmgPcnTetSulTmp	47	40	4	10	13	2
AmgPhnTetSulTmp	2	1	0	0	0	0
AmgPhnTmp	1	0	0	0	0	0
AmgSul	6	2	0	1	0	0
AmgSulTmp	5	2	0	2	0	0
AmgTet	3	3	1	0	0	0
AmgTetSul	7	6	1	3	3	0
AmgTetSulTmp	13	20	0	2	1	1
AmgTetTmp	0	1	0	0	0	0
AmgTmp	3	0	0	0	0	1
Ceph	0	1	0	1	0	0
CephAmg	0	1	0	0	0	0
CephAmgFqPhnSulTmp	0	1	0	0	0	0
CephAmgFqPhnTetSulTmp	0	1	0	0	0	0
CephAmgFqTetSul	0	0	0	1	0	0
CephAmgPcn	0	0	1	0	0	0
CephAmgPcnAmcTetSulTmp	1	0	0	0	0	0
CephAmgPcnFqPhnTetSulTmp	0	1	0	0	0	0
CephAmgPcnFqSulTmp	1	0	0	0	0	0

CephAmgPcnFqTetSulTmp	2	1	0	0	0	0
CephAmgPcnPhnTetSul	0	1	0	0	0	0

Antibiogram	Human	Poultry	Bovine	Goat	Pig	Rabbit
CephAmgPcnTetSul	0	1	0	0	0	0
CephAmgPcnTetSulTmp	2	4	1	0	1	0
CephAmgTet	0	0	0	1	0	0
CephAmgTetSulTmp	0	3	0	0	0	0
CephPcnAmcFqTetTmp	1	0	0	0	0	0
CephPcnFqPhnSulTmp	1	0	0	0	0	0
CephPcnFqTetSulTmp	1	0	0	0	0	0
CephPcnSulTmp	0	0	0	1	0	0
CephPcnTetSulTmp	0	0	0	1	0	0
CephSul	0	1	0	0	0	0
CephTetSulTmp	0	2	1	0	0	0
Fq	3	1	0	0	0	0
FqSul	0	1	0	0	0	0
FqSulTmp	1	2	0	1	0	0
FqTet	0	0	0	0	1	0
FqTetSul	1	0	0	0	0	0
FqTetSulTmp	2	4	0	0	0	1
FqTmp	1	0	0	0	0	0
Pcn	2	1	0	2	2	0
PcnAmc	1	0	0	0	0	0
PcnAmcFqTetSulTmp	0	0	0	0	0	1
PcnAmcTetSulTmp	1	0	0	0	0	0
PcnFq	1	0	0	0	0	0
PcnFqTetSulTmp	2	0	0	0	0	0
PcnPhnTet	1	0	0	1	0	0
PcnPhnTetSulTmp	1	3	0	0	0	0
PcnSul	0	1	0	0	0	0
PcnSulTmp	6	3	0	0	0	0
PcnTet	0	2	0	2	0	0
PcnTetSul	2	1	0	0	0	0
PcnTetSulTmp	9	13	2	2	3	1
PcnTetTmp	1	0	0	0	0	0
Phn	0	0	0	0	0	1
PhnSul	0	1	0	0	0	0
PhnSulTmp	1	0	0	1	0	0
Sul	18	18	3	11	1	4
SulTmp	23	17	3	0	0	0
Tet	7	12	0	2	0	0
TetSul	0	6	1	1	0	0
TetSulTmp	11	51	4	5	5	2
TetTmp	1	1	0	0	0	1

Tmp	2	2	0	0	0	0

Profile composition and number of *E. coli* isolates from human and animal in Nairobi, Kenya. Abbreviations: Cephalosporins (Ceph), Aminoglycosides (Amg), Phenicols (Phn), Tetracyclines (Tet), Penicillins (Pcn), Penicillin lactams (Amc), Folate inhibitors (Tmp), Sulfonamides (Sul) and Fluoroquinolones (Fq).

Appendix C for: One Health genomic epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in Nairobi, Kenya

Appendix Table C1. Distribution of AMR gene by host type.

Antimicrobial Class	ARGs	Human (n=315)	Poultry (n=314)	Pigs (n=50)	Bovine (n=61)	Goat (n=128)	Rabbit (n=41)	p value	p.adj
Sulphonamide	***sul1***	83 (26.3)	45 (14.3)	9 (18)	8 (13.1)	13 (10.2)	5 (12.2)	0.0001	**0.01**
	sul2	171 (54.3)	169 (53.8)	26 (52)	20 (32.8)	30 (23.4)	7 (17.1)	<0.001	**<0.001**
	sul3	2 (0.6)	14 (4.5)	1 (2)	1 (1.6)	0	0	0.006	0.4
Aminoglycoside	*strA*	160 (50.8)	144 (45.9)	27 (54)	17 (27.9)	27 (21.1)	5 (12.2)	<0.001	**<0.001**
	strB	159 (50.5)	146 (46.5)	26 (52)	17 (27.9)	27 (21.1)	5 (12.2)	<0.001	**<0.001**
	aadA1	41 (13)	37 (11.8)	5 (10)	4 (6.6)	8 (6.2)	5 (12.2)	0.33	1
	aadA2	7 (2.2)	15 (4.8)	0	1 (1.6)	3 (2.3)	0	0.17	1
	aadA24	1 (0.3)	1 (0.3)	0	0	0	0	0.97	1
	aadA4	1 (0.3)	0	0	0	0	0	0.86	1
	aadA5	19 (6)	24 (7.6)	1 (2)	3 (4.9)	3 (2.3)	1 (2.4)	0.21	1
	aac3_IIa	1 (0.3)	0	0	0	0	0	0.86	1
	aac3_IId	3 (1)	2 (0.6)	0	0	0	0	0.77	1
	aph3_Ia	1 (0.3)	2 (0.6)	1 (2)	0	1 (0.8)	1 (2.4)	0.5	1
	aph3_Ic	0	2 (0.6)	0	0	0	0	0.58	1
	aph6_Ic	0	0	0	0	1 (0.8)	0	0.3	1
Tetracycline	***tetA***	113 (35.9)	161 (51.3)	25 (50)	15 (24.6)	21 (16.4)	10 (24.4)	<0.001	**<0.001**
	tetB	31 (9.8)	17 (5.4)	6 (12)	2 (3.3)	9 (7)	1 (2.4)	0.1	1
	tetD	0	0	0	0	0	1 (2.4)	0.0008	0.06
β-lactams	*blaCARB_2*	1 (0.3)	0	0	0	0	0	0.86	1
	blaCMY_48	1 (0.3)	0	0	0	0	0	0.86	1
	blaCMY_60	1 (0.3)	0	0	0	0	0	0.86	1
	blaCMY_68	1 (0.3)	0	0	0	0	0	0.86	1
	blaTEM_1B	108 (34.3)	67 (21.3)	21 (42)	11 (18)	16 (12.5)	4 (9.8)	<0.001	**<0.001**
	blaCTX_M_15	7 (2.2)	2 (0.6)	1 (2)	0	0	0	0.2	1
	blaTEM_33	1 (0.3)	0	0	0	0	0	0.86	1
	blaCMY_79	1 (0.3)	0	0	0	0	0	0.86	1
	blaCTX_M_14	1 (0.3)	1 (0.3)	0	0	0	0	0.97	1
	blaOXA_1	7 (2.2)	1 (0.3)	0	0	3 (2.3)	0	0.16	1
	blaTEM_1C	6 (1.9)	9 (2.9)	0	0	1 (0.8)	1 (2.4)	0.45	1
	blaOXA_10	1 (0.3)	0	0	0	1 (0.8)	0	0.7	1
	blaSHV_1	1 (0.3)	0	0	0	0	0	0.86	1
	blaTEM_176	0	2 (0.6)	0	0	0	0	0.58	1
	blaTEM_207	0	0	1 (2)	0	0	0	0.004	0.3
	blaTEM_30	2 (0.6)	0	0	0	0	0	0.58	1
	ampC	1 (0.3)	0	0	0	0	0	0.86	1
Macrolide	*mphA*	27 (8.6)	11 (3.5)	2 (4)	1 (1.6)	4 (3.1)	1 (2.4)	0.02	1
Trimethoprim	***dfrA1***	114 (36.2)	146 (46.5)	16 (32)	15 (24.6)	15 (11.7)	7 (17.1)	<0.001	**<0.001**
	dfrA12	5 (1.6)	13 (4.1)	0	1 (1.6)	3 (2.3)	0	0.2	1
	dfrA14	54 (17.1)	87 (27.7)	15 (30)	8 (13.1)	7 (5.5)	2 (4.9)	<0.001	**<0.001**
	dfrA7	46 (14.6)	16 (5.1)	4 (8)	3 (4.9)	5 (3.9)	1 (2.4)	0.0001	**0.005**
	dfrA8	17 (5.4)	4 (1.3)	1 (2)	0	1 (0.8)	0	0.005	0.36
	dfrA15	3 (1)	0	0	0	0	0	0.34	1
	dfrA16	1 (0.3)	0	0	0	0	0	0.86	1
	dfrA17	19 (6)	24 (7.6)	1 (2)	3 (4.9)	3 (2.3)	1 (2.4)	0.21	1

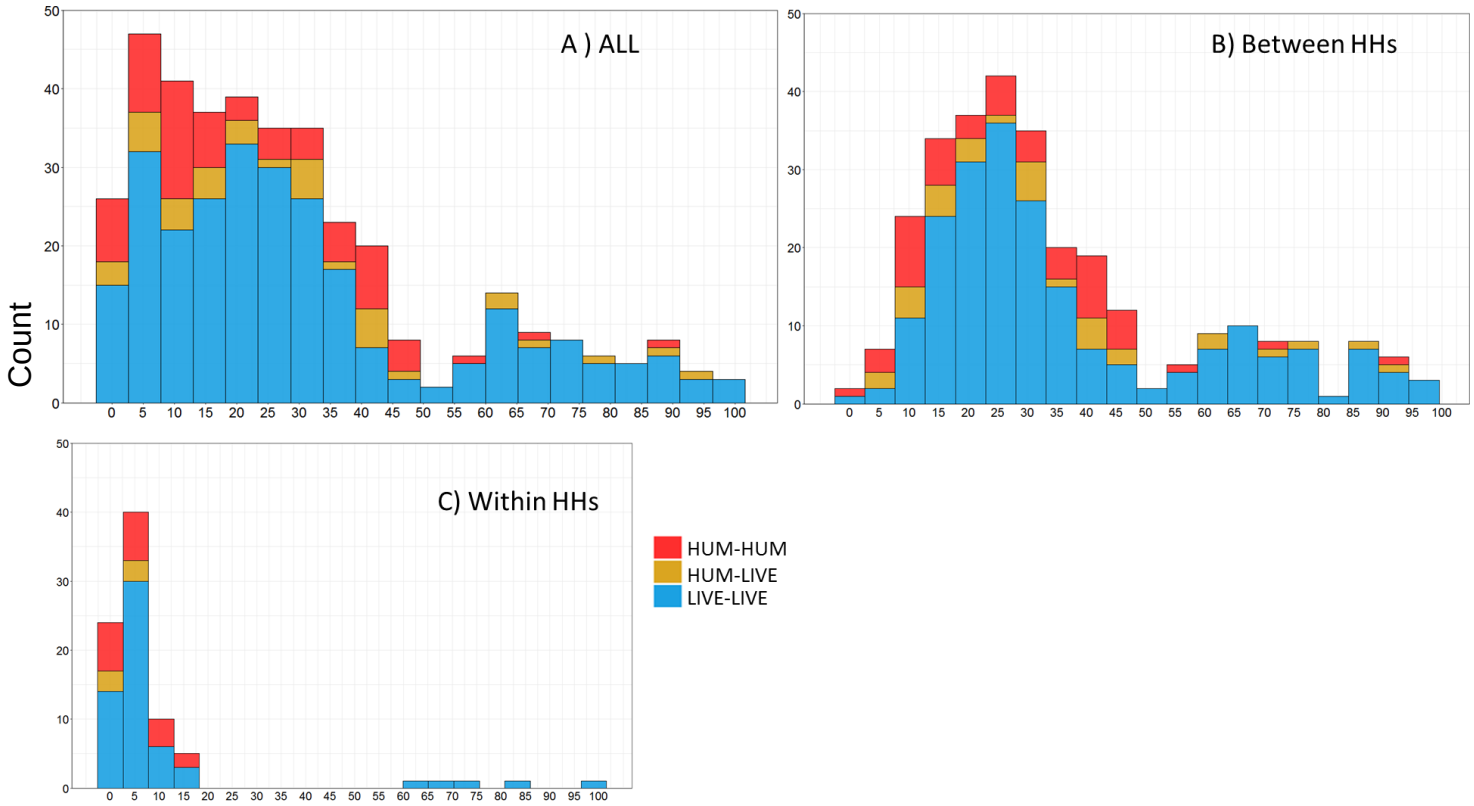
	dfrA21	1 (0.3)	0	0	0	0	0	0.86	1
	dfrA5	14 (4.4)	9 (2.9)	4 (8)	0	4 (3.1)	2 (4.9)	0.27	1
	dfrB4	1 (0.3)	0	0	0	0	0	0.86	1
Fluoroquinolones	*qepA*	2 (0.6)	1 (0.3)	0	0	1 (0.8)	0	0.93	1
	qnrB19	1 (0.3)	1 (0.3)	0	0	0	0	0.97	1
	qnrB2	0	1 (0.3)	0	0	1 (0.8)	0	0.7	1
	qnrB60	1 (0.3)	0	0	0	0	0	0.86	1
	qnrB7	0	1 (0.3)	0	0	0	0	0.86	1
	qnrS1	15 (4.8)	10 (3.2)	4 (8)	1 (1.6)	2 (1.6)	0	0.15	1
	qnrS2	0	1 (0.3)	0	0	0	0	0.86	1
	aac6Ib_cr	2 (0.6)	0	0	0	0	0	0.58	1
	gyrA_D87Y	1 (0.3)	0	0	0	0	0	0.86	1
	gyrA_S83L	28 (8.9)	14 (4.5)	2 (4)	0	1 (0.8)	1 (2.4)	0.002	0.16
	gyrA_S83A	2 (0.6)	0	0	0	0	0	0.58	1
	gyrA_D87N	7 (2.2)	4 (1.3)	0	0	1 (0.8)	1 (2.4)	0.59	1
	parC_E84A	1 (0.3)	0	0	0	0	0	0.86	1
	parC_E84V	2 (0.6)	0	0	0	0	0	0.58	1
	parC_A56T	2 (0.6)	9 (2.9)	3 (6)	1 (1.6)	1 (0.8)	0	0.05	1
	parC_S57T	2 (0.6)	2 (0.6)	0	0	0	0	0.88	1
	parC_A108V	0	1 (0.3)	0	0	0	0	0.86	1
	parC_S80I	9 (2.9)	6 (1.9)	0	0	1 (0.8)	1 (2.4)	0.46	1
	parC_E84G	1 (0.3)	1 (0.3)	0	0	0	0	0.97	1
	parE_I529L	4 (1.3)	0	0	0	0	0	0.18	1
	parE_D475E	4 (1.3)	3 (1)	0	0	1 (0.8)	0	0.86	1
Phenicols	***catA1***	32 (10.2)	5 (1.6)	1 (2)	1 (1.6)	2 (1.6)	0	<0.001	**0.0001**
	catA2	2 (0.6)	2 (0.6)	0	1 (1.6)	0	0	0.76	1
	catB3	2 (0.6)	0	0	0	0	0	0.58	1
	cmlA1	2 (0.6)	7 (2.2)	0	0	1 (0.8)	0	0.3	1
	floR	0	1 (0.3)	0	0	0	0	0.86	1
Fosfomycin	*fosA*	0	3 (1)	1 (2)	0	0	0	0.21	1

Table C2: Results of separate Poisson generalised linear mixed models investigating household risk factors for individual AMR genes in humans at the household level. Households not keeping livestock and having no manure in the household were used as the reference level in all models. Only included gene having a prevalence of greater or equal to 15%.

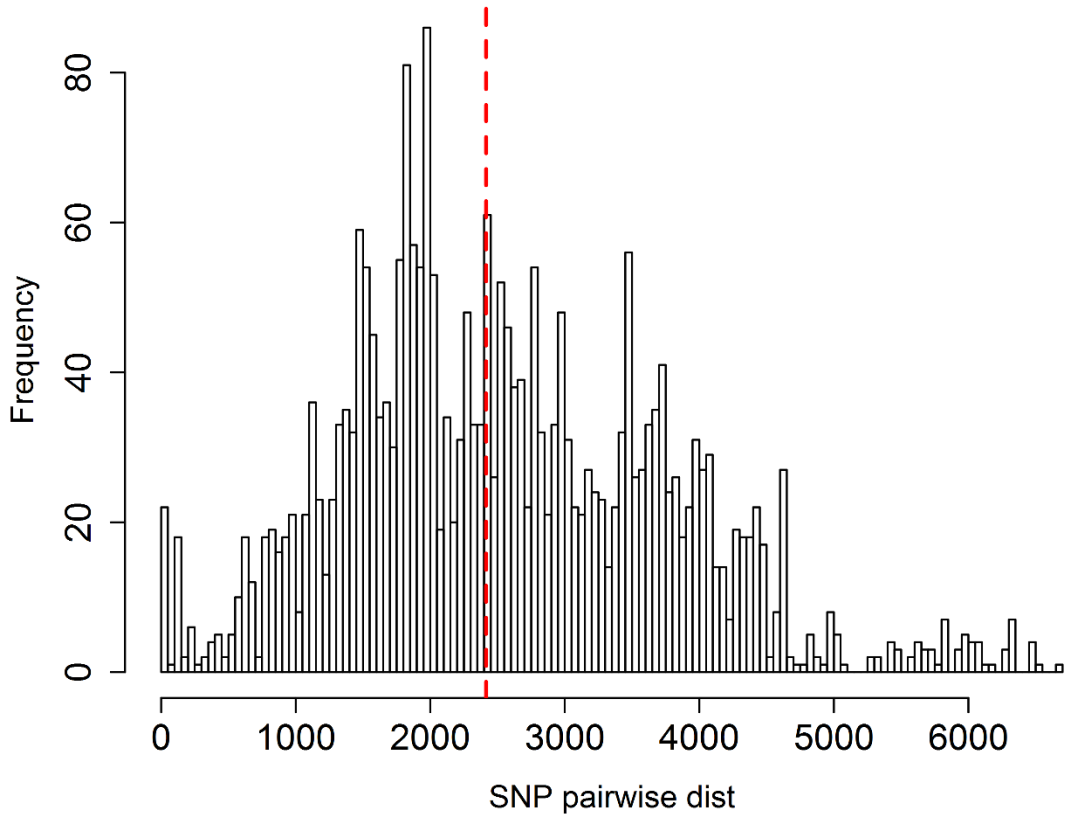
Model 1: AMR gene length, humans in all households	**Estimate**	**SE**	**P value**
sul2			
Human density	0.5827	0.22279	0.00891
Large livestock (+/- small livestock)	-0.03187	0.32072	0.92083
Small livestock only	0.48931	0.30248	0.10573
strA			
Human density	0.58846	0.21411	0.00599
Large livestock (+/- small livestock)	0.07125	0.31414	0.82057
Small livestock only	0.30505	0.29455	0.30037
strB			
Human density	0.62736	0.20997	0.00281
Large livestock (+/- small livestock)	0.07983	0.30766	0.79526
Small livestock only	0.27992	0.28873	0.3323
dfrA1			
Human density	0.0944	0.19973	0.6365
Large livestock (+/- small livestock)	-0.04276	0.31329	0.8914
Small livestock only	0.22329	0.28873	0.4393
tetA			
Human density	0.0944	0.19973	0.6365
Large livestock (+/- small livestock)	-0.04276	0.31329	0.8914
Small livestock only	0.22329	0.28873	0.4393
$bla_{TEM\text{-}1B}$			
Human density	0.7257	0.235	0.002015
Large livestock (+/- small livestock)	-0.5728	0.3577	0.109273
Small livestock only	-0.1068	0.3241	0.741799
dfrA14			
Human density	-0.04071	0.3246	0.9
Large livestock (+/- small livestock)	1.16133	0.53196	0.029
Small livestock only	1.13879	0.50792	0.025
Model 1: humans in livestock keeping household only			
tetA			
Human density	0.5412	0.2511	0.03112
Manure in household	0.0596	0.3346	0.85863
sul2			
Human density	0.5772	0.2675	0.0309
Manure in household	0.5573	0.3329	0.0941
strA			
Human density	0.6237	0.2592	0.01613
Manure in household	0.5017	0.3198	0.11667
strB			
Human density	0.6237	0.2592	0.01613
Manure in household	0.5017	0.3198	0.11667
dfrA1			
Human density	0.1275	0.2472	0.606
Manure in household	0.1288	0.329	0.695
dfrA14			
Human density	-0.12502	0.3936	0.7508
Manure in household	-0.03976	0.4653	0.9319
$bla_{TEM\text{-}1B}$			
Human density	0.5857	0.2849	0.0398
Manure in household	0.9288	0.421	0.0274

11.2 Appendix D for: *Escherichia coli* genetic diversity and sharing in co-habiting human and livestock populations in Nairobi, Kenya

Appendix Figure D1. Histogram of SNP pairwise distances between isolates with differences of 0-100 core genome SNPs coloured by type of sharing (A) All isolates (B) isolates involved in between-household sharing only, and (C) isolates involved in within-household sharing only.



Appendix Figure D2. Histogram of SNP pairwise distances between isolates belonging to sequence type 10. The distributions represent 2628 pairwise comparisons. Red line represents the median of the pairwise distance.



Appendix Table D1. Summary of metadata associated with isolates involved in human-livestock sharing

Pair	Isolates	Host type	Pairwise SNPs	ST	Household ID	Wealth grp	AMR genes	Role in HH (for humans)	Livestock keeping
1	ALQ020141	Human	3	23	UTH30	6	*bla*$_{TEM-1B}$,*dfrA8,mph(A),strA,strB,sul2*	Male	No
	ALQ020449	Poultry (Chicken)		23	UTH29	6	*blaTEM-1B,dfrA8,mph(A),strA,strB,sul2*		Yes
2	TMP024272	Human	7	48	LOR099	1	*blaTEM-1C*	Male - head - no livestock contact - bachelor's degree	No
	ALQ023279	Goat		48	SPV064	2	*bla*$_{TEM-1C}$,*tet(A)*		Yes
3	ALQ023299	Human	2	NVL	SPV064	2		Male - Farmhand - handling - manure - milking - collecting eggs - slaughter- primary school	Yes
	INT006051	Rabbit		NVL	SPV064	2			Yes
4	ALQ023299	Human	4	NVL	SPV064	2		Male - Farmhand - handling - manure - milking - collecting eggs - slaughter- primary school	Yes
	INT006147	Poultry (Turkey)		NVL	SPV064	2			Yes
5	INT004723	Human	3	6178	KRR037	2		Male - head - handling - milking -collecting eggs - bachelor's degree	Yes
	INT004696	Bovine		6178	KRR037	2			Yes
6	INT007533	Human	2	58	MWK056	6		Male - head - handling - manure -diploma	Yes
	INT007394	Poultry (Duck)		58	MWK056	6	*strA,strB,sul2,tet(A)*		Yes
7	TMP022790	Human	8	10	NGN080	5		Male - Farmhand - handling - manure - milking - collecting eggs - slaughter-primary school	Yes
	INT008356	Poultry (Chicken)		10	GTN074	6			Yes
8	PLQ2189	Human	6	538	KTS088	1		Male - Farmhand - handling - manure - milking - collecting eggs - slaughter-primary school	Yes
	PLQ2296	Poultry (Goose)		538	KTS088	1			Yes
9	TMP021689	Human	0	206	KHW049	6	*aadA1,aph(3')-Ia,dfrA14,mph(A),strA,strB,sul2,tet(A)*	Male.	Yes
	TMP021678	Poultry		206	KHW049	6	*aadA1,aph(3')-Ia,dfrA14, tet(A), mph(A),strA,strB,sul2*		Yes

Appendix Table D2. Summary of metadata of isolates involved in sharing. *BOV – Bovine, HUM – Human, RAB – Rabbit and POL – Poultry.

Pair	SNP distance	Household combination	Host combination*	Wealth	ST	Keeping livestock	ARGs
INT005621-TMP019524	7	KHW049-KIG020	BOV-BOV	6	ST906	Yes	
INT005621-TMP021809	6	KHW049	BOV-BOV	6	ST906	Yes	
TMP016878-TMP016889	5	KAN007	BOV-BOV	1	ST187	Yes	
INT004696-INT004723	3	KRR037	BOV-HUM	2	ST6178	Yes	
TMP016016-TMP019096	8	MSA004-MAK017	BOV-RAB	6	ST155	Yes	*aadA1,blaTEM1B,dfrA1,sul1,sul2,tetA -- dfrA5,strA,strB,sul2,tetA*
ALQ017189-INT005029	4	KIL042	GOAT	2	ST297	Yes	
ALQ019065-ALQ019380	9	KIG019	GOAT	6	ST223	Yes	*PanSuc -- dfrA5,strA,strB,sul1,sul2*
ALQ019365-TMP019339	9	KIG019	GOAT	6	NVL	Yes	
ALQ020209-ALQ020457	0	UTH029	GOAT	6	ST1079	Yes	
INT004567-INT007541	9	UMJ036-MWK057	GOAT	4-6	ST297	Yes	
INT004567-INT007592	8	UMJ036-MWK057	GOAT	4-6	ST297	Yes	
INT005034-INT005045	3	KIL042	GOAT	2	ST3884	Yes	
INT007541-INT007592	1	MWK057	GOAT	6	ST297	Yes	
PLQ000563-PLQ000593	7	NGN079	GOAT	5	ST4088	Yes	
TMP014928-TWAG1869	9	VIW002-BOM092	GOAT	7-3	ST297	Yes	*aadA1,blaTEM1B,dfrA1,strA,strB,sul1,sul2,tetA -- PanSuc*
TMP014928-TWAG1878	8	VIW002-BOM092	GOAT	7-3	ST297	Yes	*aadA1,blaTEM1B,dfrA1,strA,strB,sul1,sul2,tetA -- PanSuc*
TMP019846-TMP019851	8	MLK023	GOAT	7	ST223	Yes	
TMP020486-TMP020499	6	KWG070	GOAT	6	ST56	Yes	*PanSuc -- strA,strB,sul2*
TMP020486-TMP020669	2	KWG070	GOAT	6	ST56	Yes	
TMP020499-TMP020669	6	KWG070	GOAT	6	ST56	Yes	*strA,strB,sul2 -- PanSuc*
TWAG1869-TWAG1878	3	BOM092	GOAT	3	ST297	Yes	

Appendix Table D2. Summary of metadata of isolates involved in sharing. *BOV – Bovine, HUM – Human, RAB – Rabbit and POL – Poultry.

Pair	SNP distance	Household combination	Host combination*	Wealth	ST	Keeping livestock	ARGs
INT005621-TMP019524	7	KHW049-KIG020	BOV-BOV	6	ST906	Yes	
INT005621-TMP021809	6	KHW049	BOV-BOV	6	ST906	Yes	
TMP016878-TMP016889	5	KAN007	BOV-BOV	1	ST187	Yes	
INT004696-INT004723	3	KRR037	BOV-HUM	2	ST6178	Yes	
TMP016016-TMP019096	8	MSA004-MAK017	BOV-RAB	6	ST155	Yes	*aadA1,blaTEM1B,dfrA1,sul1,sul2,tetA -- dfrA5,strA,strB,sul2,tetA*
ALQ017189-INT005029	4	KIL042	GOAT	2	ST297	Yes	
ALQ019065-ALQ019380	9	KIG019	GOAT	6	ST223	Yes	*PanSuc -- dfrA5,strA,strB,sul1,sul2*
ALQ019365-TMP019339	9	KIG019	GOAT	6	NVL	Yes	
ALQ020209-ALQ020457	0	UTH029	GOAT	6	ST1079	Yes	
INT004567-INT007541	9	UMJ036-MWK057	GOAT	4-6	ST297	Yes	
INT004567-INT007592	8	UMJ036-MWK057	GOAT	4-6	ST297	Yes	
INT005034-INT005045	3	KIL042	GOAT	2	ST3884	Yes	
INT007541-INT007592	1	MWK057	GOAT	6	ST297	Yes	
PLQ000563-PLQ000593	7	NGN079	GOAT	5	ST4088	Yes	
TMP014928-TWAG1869	9	VIW002-BOM092	GOAT	7-3	ST297	Yes	*aadA1,blaTEM1B,dfrA1,strA,strB,sul1,sul2,tetA -- PanSuc*
TMP014928-TWAG1878	8	VIW002-BOM092	GOAT	7-3	ST297	Yes	*aadA1,blaTEM1B,dfrA1,strA,strB,sul1,sul2,tetA -- PanSuc*
TMP019846-TMP019851	8	MLK023	GOAT	7	ST223	Yes	
TMP020486-TMP020499	6	KWG070	GOAT	6	ST56	Yes	*PanSuc -- strA,strB,sul2*
TMP020486-TMP020669	2	KWG070	GOAT	6	ST56	Yes	
TMP020499-TMP020669	6	KWG070	GOAT	6	ST56	Yes	*strA,strB,sul2 -- PanSuc*
TWAG1869-TWAG1878	3	BOM092	GOAT	3	ST297	Yes	
ALQ023279-TMP024272	7	SPV064-LOR099	GOAT-HUM	2-1	ST48	Yes-No	*blaTEM1C,tetA -- blaTEM1C*
ALQ020462-INT010077	1	UTH029-SPV100	GOAT-RAB	6-2	ST7324	Yes	
ALQ019547-ALQ019550	1	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*
ALQ019547-ALQ019554	6	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*

Pair	SNP distance	Household combination	Host combination*	Wealth	ST	Keeping livestock	ARGs
ALQ019547-TMP018942	9	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*
ALQ019550-ALQ019554	7	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*
ALQ019550-TMP018942	8	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*
ALQ019554-TMP018942	3	MLK024	HUM	7	ST678	Yes	*blaTEM1B,catA1,dfrA7,dfrA8,mphA,strA,strB,sul2,tetA*
ALQ019662-ALQ019663	0	KYL025	HUM	6	ST155	Yes	*blaTEM1B,dfrA14,strA,strB,sul2,tetA -- PanSuc*
ALQ019705-ALQ019713	8	KYL027	HUM	6	ST2351	No	
ALQ019705-ALQ019749	7	KYL027	HUM	6	ST2351	No	
ALQ019705-ALQ019784	8	KYL027	HUM	6	ST2351	No	
ALQ019713-ALQ019784	0	KYL027	HUM	6	ST2351	No	
ALQ019911-ALQ019980	7	KYL026-UTH028	HUM	6	ST10	Yes	*catA1,dfrA7,strA,strB,sul1,sul2,tetA*
ALQ032869-ALQ034796	5	MUT084-EMB086	HUM	2-4	ST69	Yes-No	*aac3-IIa,dfrA14,QnrS1,strA,strB,sul2 -- blaTEM1B*
ALQ034735-PLQ2415	4	EMB086-KTS089	HUM	4-1	ST10	No-Yes	*strA,strB,sul2,tetA*
INT003900-INT004245	1	DAN032	HUM	6	ST10	Yes	*blaTEM1B,dfrA7,strA,strB,sul1,sul2*
INT004422-INT004425	0	UMJ034	HUM	4	ST131	No	*aadA5,blaTEM1B,dfrA17,mphA,strA,strB,sul1,sul2 -- PanSuc*
INT004642-INT004645	7	KRR037	HUM	2	ST1611	Yes	
INT005269-PLQ2349	9	EAS045-KTS089	HUM	5-1	ST1136	No-Yes	*aadA1,dfrA1,strA,strB,sul2,tetA*
INT008624-INT008641	4	EMB086	HUM	4	ST216	No	*PanSuc -- blaTEM1B,dfrA8,strA,strB,sul2*
PLQ2481-TMP016360	2	KTS089-HAR010	HUM	1-3	ST210	Yes	*dfrA5,mphA,sul1*
TMP018696-TMP019915	2	MLK023	HUM	7	NVL	Yes	
TMP019182-TMP019284	7	KIG019	HUM	6	ST10	Yes	*aadA1,blaTEM1B,catA1,dfrA1,strA,strB,sul1,sul2*
TMP019273-TMP019279	1	KIG019	HUM	6	ST131	Yes	*blaTEM-30,mphA,strA,strB,sul2*
ALQ020141-ALQ020449	3	UTH030-UTH029	HUM-POL	6	ST23	No-Yes	*blaTEM1B,dfrA8,mphA,strA,strB,sul2*
ALQ023299-INT006147	4	SPV064	HUM-POL	2	NVL	Yes	
PLQ2189-PLQ2296	6	KTS088	HUM-POL	1	ST538	Yes	
ALQ023299-INT006051	2	SPV064	HUM-RAB	2	NVL	Yes	
ALQ028389-INT007544	10	KOR059-MWK057	PIG	7-6	ST48	Yes	*blaTEM1B,dfrA5,strA,strB,sul2,tetA*
TMP018094-TMP018438	3	KYL026	PIG	6	ST1302	Yes	
ALQ017153-ECL000675	5	KIL042	POL	2	ST6635	Yes	
ALQ017241-ALQ017368	5	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*
ALQ017241-TMP016254	6	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*
ALQ017241-TMP016275	4	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*

Pair	SNP distance	Household combination	Host combination*	Wealth	ST	Keeping livestock	ARGs
ALQ017368-TMP016254	5	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*
ALQ017368-TMP016275	5	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*
ALQ018881-ALQ019005	9	MAK017	POL	6	ST2309	Yes	*dfrA14,strA,strB,sul2*
ALQ018914-ALQ019107	8	MAK017	POL	6	ST3333	Yes	*dfrA14,strA,strB,sul2,tetA -- PanSuc*
ALQ034646-TMP023415	4	EMB085	POL	4	ST223	Yes	
ALQ034661-TMP023356	1	EMB085	POL	4	ST2622	Yes	
ALQ034843-ALQ034865	1	EMB087	POL	4	ST1638	Yes	*dfrA14,strA,strB,sul2,tetA*
INT003119-INT003176	3	KRR037	POL	2	ST1302	Yes	*PanSuc -- sul2*
INT003875-INT003892	2	DAN032	POL	6	ST4162	Yes	*blaTEM1B,dfrA8,strA,strB,sul2,tetB*
INT004680-INT004683	1	KRR037	POL	2	ST5273	Yes	*strA,strB,sul2,tetA*
INT005129-INT005135	3	EAS043	POL	5	ST48	Yes	*dfrA14,QnrS1,strA,strB,sul2,tetA*
PLQ000871-PLQ000881	4	MUT082	POL	2	ST746	Yes	*dfrA14,strA,strB,sul2,tetA*
PLQ2453-PLQ2462	1	KTS089	POL	1	ST162	Yes	
PLQ2453-PLQ2468	2	KTS089	POL	1	ST162	Yes	
PLQ2462-PLQ2468	3	KTS089	POL	1	ST162	Yes	
TMP016254-TMP016275	4	HAR011	POL	3	ST1196	Yes	*aadA1,aadA2,cmlA1,dfrA12,sul3*
TMP023349-TMP023446	0	EMB085	POL	4	ST189	Yes	*dfrA14,strA,strB,sul2,tetA*
TWAG1723-TWAG1772	4	BOM091	POL	3	ST2351	Yes	
PLQ000667-TMP022768	6	NGN080	POL-GOAT	5	ST2614	Yes	*aadA5,dfrA17,sul2,tetA*
TMP015782-TMP021071	3	VIW001-GTN074	POL-GOAT	7-6	ST155	Yes	*blaTEM1B,dfrA5,strA,strB,sul2,tetA*
INT007394-INT007533	2	MWK056	POL-HUM	6	ST58	Yes	*strA,strB,sul2,tetA -- PanSuc*
INT008356-TMP022790	8	GTN074-NGN080	POL-HUM	6-5	ST10	Yes	
TMP021678-TMP021689	0	KHW049	POL-HUM	6	ST206	Yes	*aadA1,aph3''-Ia,dfrA14,mphA,strA,strB,sul2,tetA*
ALQ034833-INT008582	6	EMB087	POL-RAB	4	ST1125	Yes	
ALQ017321-ALQ017332	6	HAR011	RAB	3	ST4568	Yes	
ALQ017321-TMP016046	6	HAR011	RAB	3	ST4568	Yes	
ALQ017321-TMP016335	5	HAR011	RAB	3	ST4568	Yes	
ALQ017332-TMP016046	0	HAR011	RAB	3	ST4568	Yes	
ALQ017332-TMP016335	5	HAR011	RAB	3	ST4568	Yes	*PanSuc -- aadA1,dfrA1,sul1,tetA*
TMP016046-TMP016335	5	HAR011	RAB	3	ST4568	Yes	*PanSuc -- aadA1,dfrA1,sul1,tetA*
TWAG00324-TWAG00337	1	MIH068	RAB	5	ST1304	Yes	
TWAG00324-TWAG00343	2	MIH068	RAB	5	ST1304	Yes	

Pair	SNP distance	Household combination	Host combination*	Wealth	ST	Keeping livestock	ARGs
TWAG00337-TWAG00343	1	MIH068	RAB	5	ST1304	Yes	
INT006051-INT006147	4	SPV064	RAB-POL	2	NVL	Yes	
TWAG1754-TWAG1761	10	BOM091	RAB-POL	3	ST48	Yes	*aadA1,bla*$_{TEM1C}$*,dfrA1,sul1,tetA*

11.3 Appendix E for: A cross-sectional survey of practices and knowledge among antimicrobial retailers in Nairobi, Kenya

Appendix Table E1: Summary of variables

Theme	Data collected	Data description
Socio-demographic variables	Type of drug store	• Human • Veterinary
	Age	Years
	Gender	• Male • Female
	Educational level	High • Doctorate degree • Master's degree • Bachelor's degree • College (Certificate/Diploma/Higher Diploma) Low • No formal Education • Primary Education • Secondary Education
	Number of workers in the drug store	Number
	Type of business	• Sole proprietorship • Joint ownership • Chain store
	Respondents role in the drug store	• Owner • Full time employee • Part time employee
	Duration of working in the drug store	• More than 5 years • 2-4 years • Less than 2 years
	Training in medical/veterinary sciences	• Bachelor's degree (Medicine, pharmacy, vet medicine nursing etc.) • Diploma (clinical medicine, pharmacy, animal health etc.) • Certificate (clinical medicine, pharmacy, nursing etc.) • None
	Received specific training on appropriate use of antimicrobials	Categorical (yes/no)
	Source of training	• Degree/diploma training more than 3 years ago • Degree/diploma training less than 3 years ago • Continuous development program/workshop (> 3 years ago) • Continuous development program/workshop (< 3 years ago)
Antimicrobials sold and sale dynamics	Antimicrobials available in the store for sale	20 antimicrobial classes as defined by World Health Organization List of Essential Medicines
	Common sold antimicrobial classes (top 4)	Participants were asked to subjectively describe which 4 antimicrobial classes were most commonly sold
	Antimicrobial sale changes compared to similar period in the previous year	• Increased • Decreased • No change
	Reasons for change in antimicrobial sales	• More/less demand from customers • More/less institutional procurement procedures • More/less supply coming from suppliers
Antimicrobial supply	Sources of antimicrobials	• Neighbouring wholesaler • Wholesaler in a another location in Nairobi county

		• Wholesaler in a another location outside Nairobi county • Drug distribution company • Drug manufacturing company • Import from an international source
	Important factors when purchasing antimicrobials	• Price • Service (i.e. reliability) • Product quality (trusted brand)
Antimicrobial prescribing practices and customer characteristics	Number of customers purchasing antimicrobials	Number
	Number of customers purchasing antimicrobials without a prescription	Number
	Antimicrobial classes sold without needing a written prescription	Number
	Common presented complaints for purchasing antimicrobials (human drug stores)	
	Commonly sold antimicrobials to different types of farmers/customers (veterinary drugs stores)	
	Information provided to customers when buying antimicrobials	• Correct dosage • Directions for use • Storage instructions • Potential side effects • Expiry date • Contra-indications
	Factors taken into account when recommending a certain antimicrobial to a customer	• Price of antimicrobial • Type of antimicrobial • Availability of antimicrobial • Indications of use • Efficacy • Adverse reactions/effects • Recommended antimicrobial choice • Customer preference
Knowledge on antimicrobial resistance	Statements about knowledge on antimicrobial resistance	• Antimicrobial resistance occurs when your body becomes resistant to antimicrobials and they no longer work as well • Many infections are becoming increasingly resistant to treatment by antimicrobials • If bacteria are resistant to antimicrobials, it can be very difficult or impossible to treat the infections they cause • Antimicrobial resistance is an issue that could affect me or my family • Antimicrobial resistance is only a problem for people who take antimicrobials regularly

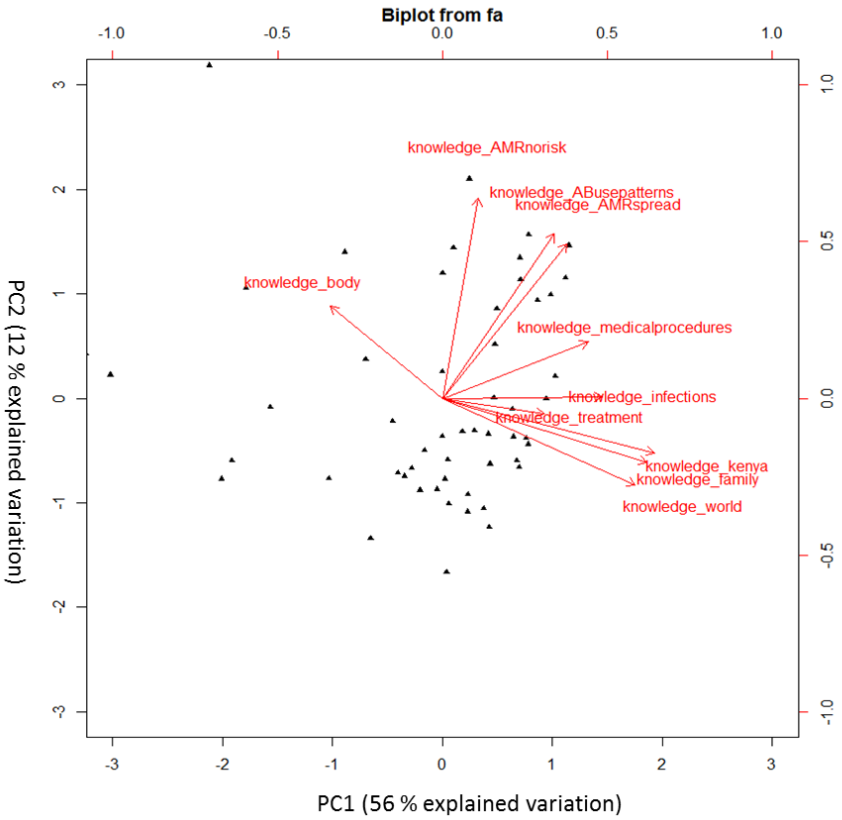
		• Bacteria which are resistant to antimicrobials can be spread from person to person • Antimicrobial-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous • Antimicrobial resistance is one of the biggest problems the world faces • Antimicrobial resistance is one of the biggest problems Kenya faces • I am not at risk of getting an antimicrobial resistant infection, as long as I take my antimicrobials correctly
	Statements about potential solutions to AMR	• There is not much people like me can do to stop antimicrobial resistance • People should use antimicrobials only when they are prescribed by a doctor or nurse • Farmers should give fewer antimicrobials to food-producing animals • People should not keep antimicrobials and use them later for other illnesses • The governments should reward the development of new antimicrobials • Pharmaceutical companies should develop new antimicrobials • Doctors should only prescribe antimicrobials when they are needed • Medical experts will solve the problem of antimicrobial resistance before it becomes too serious • Everyone needs to take responsibility for using antimicrobials responsibly • Parents should make sure all of their children's vaccinations are up-to-date • People should wash their hands regularly

Appendix Table E2. Cronbach's alpha for responses to ten statements about knowledge on antimicrobial resistance

Knowledge statement	**Raw alpha**	**Std. alpha**	**G6**	**Average R**	**S/N**	**alpha se**
Antimicrobial resistance occurs when your body becomes resistant to antimicrobials and they no longer work as well	0.74	0.75	0.81	0.25	3	0.05
Many infections are becoming increasingly resistant to treatment by antimicrobials	0.72	0.74	0.79	0.24	2.8	0.056
If bacteria are resistant to antimicrobials, it can be very difficult or impossible to treat the infections they cause	0.7	0.71	0.78	0.22	2.5	0.059
Antimicrobial resistance is an issue that could affect me or my family	0.69	0.7	0.74	0.2	2.3	0.061
Antimicrobial resistance is only a problem for people who take antimicrobials regularly	0.69	0.72	0.78	0.22	2.5	0.06
Bacteria which are resistant to antimicrobials can be spread from person to person	0.69	0.71	0.78	0.22	2.5	0.061
Antimicrobial-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous	0.69	0.71	0.78	0.22	2.5	0.06
Antimicrobial resistance is one of the biggest problems the world faces	0.69	0.7	0.76	0.2	2.3	0.06
Antimicrobial resistance is one of the biggest problems Kenya faces	0.67	0.68	0.75	0.19	2.1	0.063
I am not at risk of getting an antimicrobial resistant infection, as long as I take my antimicrobials correctly	0.73	0.75	0.8	0.25	3	0.053

* G6: Guttman's lambda 6 - calculated from the squared multiple correlation.
Average R: average inter-item correlation.

Appendix Figure E1. Principal component analysis of attitudes and perceptions related to antimicrobial resistance, from a sample of 40 human pharmacists and 19 veterinary based on the responses to ten statements about knowledge on antimicrobial resistance.



knowledge_body	Antimicrobial resistance occurs when your body becomes resistant to antimicrobials and they no longer work as well
knowledge_treatment	Many infections are becoming increasingly resistant to treatment by antimicrobials
knowledge_infections	If bacteria are resistant to antimicrobials, it can be very difficult or impossible to treat the infections they cause
knowledge_family	Antimicrobial resistance is an issue that could affect me or my family
knowledge_ABusepatterns	Antimicrobial resistance is only a problem for people who take antimicrobials regularly
knowledge_AMRspread	Bacteria which are resistant to antimicrobials can be spread from person to person
knowledge_medicalprocedures	Antimicrobial-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous
knowledge_world	Antimicrobial resistance is one of the biggest problems the world faces
knowledge_kenya	Antimicrobial resistance is one of the biggest problems Kenya faces
knowledge_AMRnorisk	I am not at risk of getting an antimicrobial resistant infection, as long as I take my antimicrobials correctly

Appendix Table E3. Percentage of responses (strongly agree/agree, strongly disagree/disagree and neutral) from 40 human and 19 veterinary pharmacists to nine statements about knowledge on antimicrobial resistance.

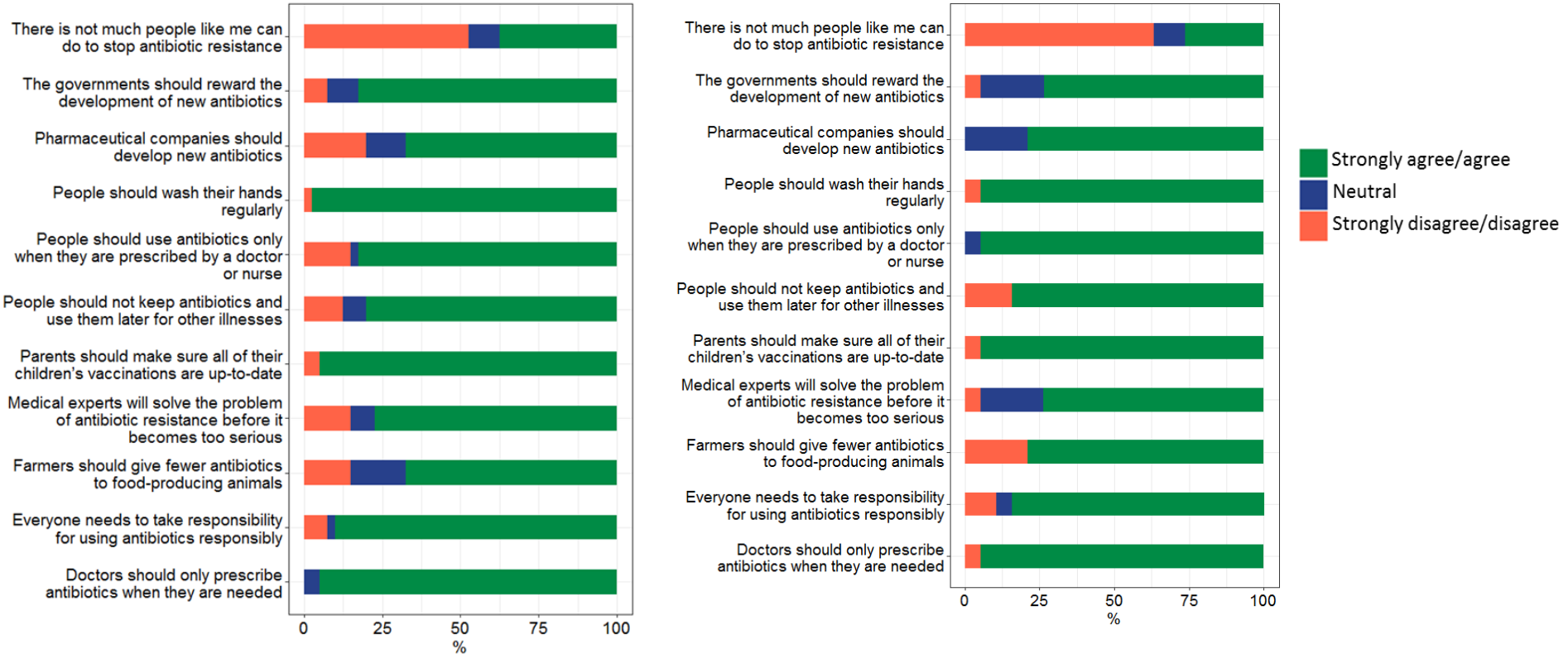
	Strongly agree/ agree		Neutral		Strongly Disagree/ Disagree	
Knowledge and awareness of AMR	Human (n=40)	Veterinary (n=19)	Human (n=40)	Veterinary (n=19)	Human (n=40)	Veterinary (n=19)
1. Antimicrobial resistance occurs when your body becomes resistant to antimicrobials and they no longer work as well	80% (32)	89.5% (17)	5% (2)	5.3% (1)	15% (6)	10.6% (2)
2. Many infections are becoming increasingly resistant to treatment by antimicrobials	87.5% (35)	89.5% (17)	5% (2)	5.3% (1)	7.5% (3)	5.3% (1)
3. If bacteria are resistant to antimicrobials, it can be very difficult or impossible to treat the infections they cause	80% (32)	79% (15)	5% (2)	5.3% (1)	15% (6)	15.8% (3)
4. Bacteria which are resistant to antimicrobials can be spread from person to person	52.5% (21)	52.7% (10)	5% (2)	26.4% (5)	42.5% (17)	21.1% (4)
5. Antimicrobial-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous	77.5% (31)	73.7% (14)	15% (6)	15.8% (3)	7.5% (3)	10.6% (2)
6. Antimicrobial resistance is one of the biggest problems the world faces	87.5% (35)	79% (15)	7.5% (3)	5.3% (1)	5% (2)	15.8% (3)
7. Antimicrobial resistance is one of the biggest problems Kenya faces	85% (34)	79% (15)	7.5% (3)	5.3% (1)	7.5% (3)	15.8% (3)
8. Antimicrobial resistance is an issue that could affect me or my family	92.5% (37)	89.5% (17)	0	0	7.5% (3)	5.3% (1)
9. Antimicrobial resistance is only a problem for people who take antimicrobials regularly	40% (16)	52.7% (10)	5% (2)	5.3% (1)	55% (22)	42.2% (8)
10. I am not at risk of getting an antimicrobial resistant infection, as long as I take my antimicrobials correctly	72.5% (29)	84.2% (16)	7.5% (3)	5.3% (1)	20% (8)	10.5% (2)

Appendix Figure E2. Percentage of responses on ten statements about knowledge on antimicrobial resistance from a) 40 human pharmacists b) 19 veterinary interviewed

Appendix Table E4. Percentage of responses (agree, disagree and neutral) from 40 human and 19 veterinary pharmacists to ten statements on potential solution to AMR.

	Strongly agree/ agree		Neutral		Strongly Disagree/ Disagree	
Perceptions about potential solutions to AMR	Human (n=40)	Veterinary (n=19)	Human (n=40)	Veterinary (n=19)	Human (n=40)	Veterinary (n=19)
1. There is not much people like me can do to stop antimicrobial resistance	37.5% (15)	26.4% (5)	10% (4)	10.6% (2)	52.5% (21)	63.2% (12)
2. People should use antimicrobials only when they are prescribed by a doctor or nurse	82.5% (33)	94.8% (18)	2.5% (1)	5.3% (1)	15% (6)	0
3. Farmers should give fewer antimicrobials to food-producing animals	67.5% (27)	79% (15)	17.5% (7)	0	15% (6)	21.1% (4)
4. People should not keep antimicrobials and use them later for other illnesses	80% (32)	84.3% (16)	7.5% (3)	0	12.5% (5)	15.8% (3)
5. The governments should reward the development of new antimicrobials	82.5% (33)	73.7% (14)	10% (4)	21.1% (4)	7.5% (3)	5.3% (1)
6. Pharmaceutical companies should develop new antimicrobials	67.5% (27)	79% (15)	12.5% (5)	21% (4)	20% (8)	0
7. Doctors should only prescribe antimicrobials when they are needed	95% (38)	94.8% (18)	5% (2)	0	0	5.3% (1)
8. Medical experts will solve the problem of antimicrobial resistance before it becomes too serious	77.5% (31)	73.7% (14)	7.5% (3)	21.1% (4)	15% (6)	5.3% (1)
9. Everyone needs to take responsibility for using antimicrobials responsibly	90% (36)	84.3% (16)	2.5% (1)	5.3% (1)	7.5% (3)	10.6% (2)
10. Parents should make sure all of their children's vaccinations are up-to-date	95% (38)	94.7% (18)	0	0	5% (2)	5.3% (1)
11. People should wash their hands regularly	97.5% (39)	94.7% (18)	0	0	2.5% (1)	5.3% (1)

Appendix Figure E3. Percentage of responses on 11 statements about potential solutions to AMR from a) 40 human b) 19 veterinary pharmacists



Appendix Table E5. Results of a multivariable regression examining the influence of sociodemographic factors on prescribing practices in a sample of 40 and 19 human and veterinary drug stores respectively.

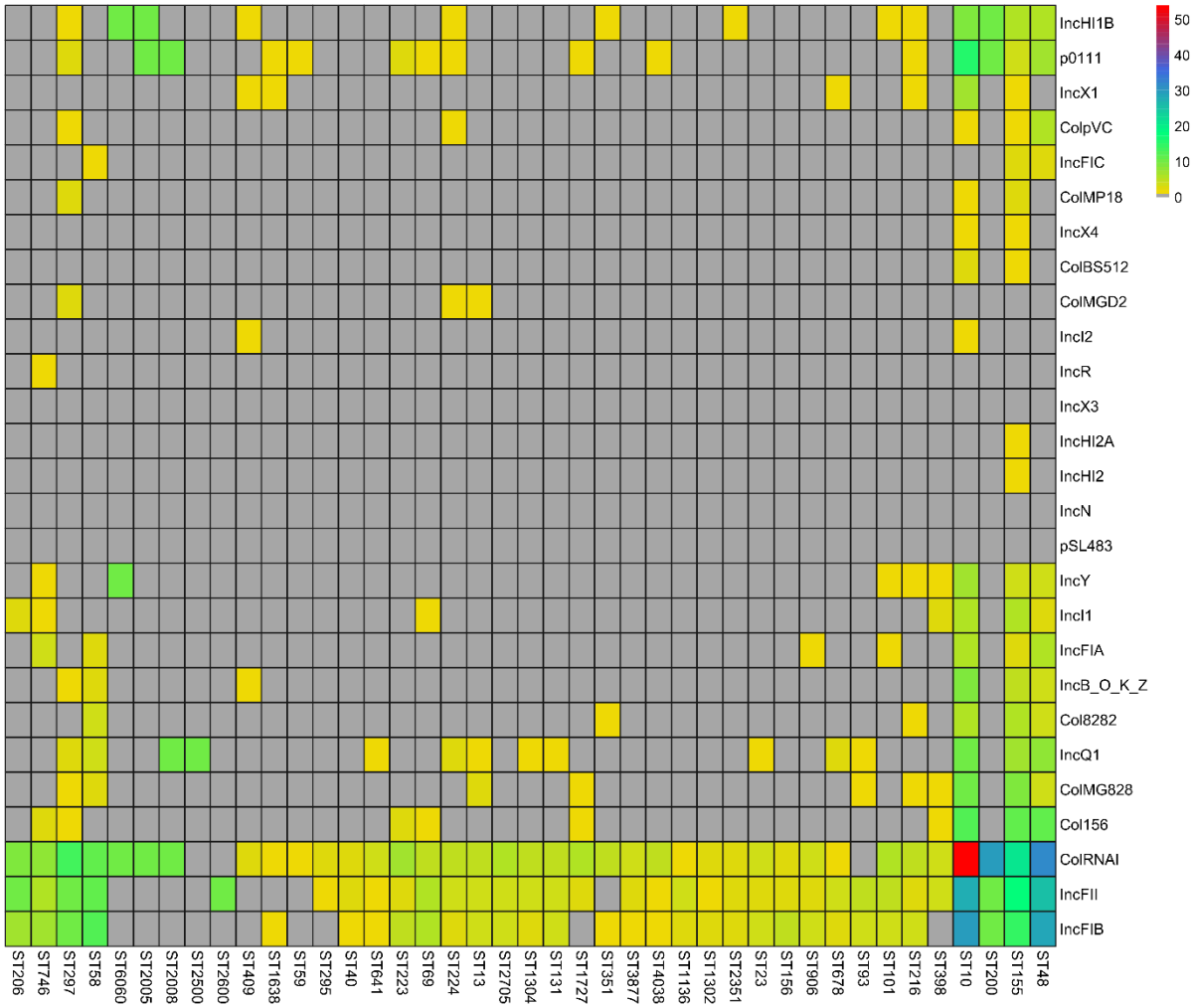
Variable	Estimate	Standard error	X^2	df	p-value
Veterinary drug store	-0.11	0.5	0.2	1	0.66
Range of antimicrobials	-0.1	0.24	0.2	1	0.62
Medical/vet training	0.38	0.25	2.4	1	0.12
High education level	-0.14	0.2	0.5	1	0.49

11.4 Appendix F for: Co-occurrence patterns of antimicrobial resistance genes and distribution of plasmids in *E. coli* isolates.

Appendix Table F1. Distribution of AMR genes among plasmid replicon types. Columns designate: number of isolates in which the replicon was identified; range (median number) of AMR genes detected in isolates with this plasmid replicon; number (%) of these isolates in which acquired AMR genes were also detected.

Replicon	#isolates	#AMR genes	ARGS (%)
ColRNAI	544	0-15 (5)	347 (63.8)
IncFII	405	0-15 (4)	245 (60.5)
IncFIB	396	0-15 (4)	237 (59.8)
IncQ1	142	0-13 (7)	141 (99.3)
p0111	142	0-15 (6)	125 (88)
Col156	130	0-13 (3)	72 (55.4)
IncHI1B	116	1-15 (5)	88 (75.9)
ColMG828	111	0-15 (2)	65 (58.6)
Not detected	78	0-10 (0)	13 (16.7)
IncFIA	77	0-13 (0)	36 (46.8)
IncY	77	0-10 (4)	45 (58.4)
IncI1	75	0-11 (2)	45 (60)
IncB_O_K_Z	75	0-13 (3)	48 (64)
Col8282	53	0-15 (6)	42 (79.2)
IncX1	45	0-10 (5)	36 (80)
ColpVC	40	0-13 (6)	31 (77.5)
ColBS512	34	0-13 (6)	28 (82.4)
IncFIC	31	0-10 (1)	17 (54.8)
ColMGD2	27	0-13 (6)	21 (77.8)
ColMP18	27	0-8 (4)	17 (63)
IncI2	19	0-11 (3)	11 (57.9)
IncR	19	0-8 (6)	16 (84.2)
IncX4	18	0-10 (1)	9 (50)
IncX3	7	1-8 (8)	7 (100)
IncN	6	7-12 (7.5)	6 (100)
IncHI2	6	8-15 (11)	6 (100)
IncHI2A	5	8-15 (10)	5 (100)
pSL483	5	0-7 (0)	2 (40)
IncX2	4	0-7 (6)	3 (75)
pEC4115	3	-	-
ColKP3	1	-	1
IncU	1	-	1

Appendix Figure F1. A heatmap illustration of associations between plasmids replicons and the most sequence types (present in at least 10 isolates)



11.5 Appendix G Study Questionnaires

Appendix Table G1. Household questionnaire, formatted for use with ODK Open Data Kit (ODK) Collect software.

label
S0Q1: Start time
S0Q2: End time
S0Q3: Date
S0Q4: Device ID
S0Q5: Simcard serial number
S0Q6: Device phone number
S0Q7: Recorder Name
S0Q8: This questionnaire is being conducted in...?
S0Q9: Scan household unique barcode on Household Master Sheet
S0Q91: The barcode has not scanned correctly. Please go back and try again or enter manually
S1Q11: Select the first letter of the current Sublocation
S1Q12: Select the current sublocation
S1Q2: Is this a livestock-owning household?
S1Q3: Select a household member to start the livestock questionnaire
S2Q1: What is the first name of the household head?
S2Q2: Which gender is ${s2q1_hh_head_id}?
S2Q3: What is the total number of family members in this household?
S2Q41: How many adult (18 and over) family members live in this household?
S2Q42: How many of the adult family members of this household are present today?
S2Q51: Is the household head present today?
S2Q52: Did ${s2q1_hh_head_id} provide a faecal sample?
S2Q53: Did ${s2q1_hh_head_id} complete a consent form?
S2Q53B: Unfortunately we cannot accept a faecal sample without a completed consent form
S2Q54: How old is ${s2q1_hh_head_id}?
S2Q55: What education has ${s2q1_hh_head_id} had?
S2Q56: What ethnicity is ${s2q1_hh_head_id}?
S2Q57: Which tribe is ${s2q1_hh_head_id}?
S2Q58: What is ${s2q1_hh_head_id}'s occupation?
S2Q59: Does ${s2q1_hh_head_id} work with any of the following outside the household?
S2Q6: Number of adult family members absent today
S2Q60: Adult family members absent today
S2Q61: What is the first name of the absent family member
S2Q62: Which gender is ${s2q61_adlt_abs_id}?
S2Q63: What relation is ${s2q61_adlt_abs_id} to ${s2q1_hh_head_id}?
S2Q64: What relation is ${s2q61_adlt_abs_id} to ${s2q1_hh_head_id}?
S2Q65: Did ${s2q61_adlt_abs_id} provide a faecal sample?
S2Q66: Did ${s2q61_adlt_abs_id} complete a consent form?
S2Q66B: Unfortunately we cannot accept a faecal sample without a completed consent form
S2Q67: How old is ${s2q61_adlt_abs_id}?
S2Q68: What education has ${s2q61_adlt_abs_id} had?
S2Q69: What ethnicity is ${s2q61_adlt_abs_id}?
S2Q610: Which tribe is ${s2q61_adlt_abs_id}?
S2Q611: What is ${s2q61_adlt_abs_id}'s occupation?
S2Q612: Does ${s2q61_adlt_abs_id} work with any of the following outside the household?
S2Q7: How many children (under 18) live in this household?
S2Q612: How many of the children in this household are present today?
S2Q8: Number of children present today
S2Q81: Number of children absent today
S2Q82: Children absent today
S2Q90: What is the first name of the absent child
S2Q91: Which gender is ${s2q91_chl_abs_id}?
S2Q92: What relation is ${s2q91_chl_abs_id} to ${s2q1_hh_head_id}?
S2Q93: What relation is ${s2q91_chl_abs_id} to ${s2q1_hh_head_id}?
S2Q99: Did ${s2q91_chl_abs_id} provide a faecal sample?
S2Q96: Did ${s2q91_chl_abs_id} complete an assent form?
S2Q96B: Unfortunately we cannot accept a faecal sample without a completed consent form
S2Q94: How old is ${s2q91_chl_abs_id}?
S2Q97: What education has ${s2q91_chl_abs_id} had?
S2Q98: What ethnicity is ${s2q91_chl_abs_id}?
S2Q910: Which tribe is ${s2q91_chl_abs_id}?
S2Q911: Does ${s2q91_chl_abs_id} attend school?
S2Q912: Does ${s2q91_chl_abs_id} eat food provided at school?
S2Q913: Does ${s2q91_chl_abs_id} eat any of these from the school?
S2: Do you employ any staff?
S2Q10: Staff employed
S2Q111: How many indoor staff (maids / cleaning staff / housekeepers / cook/nanny, etc) do you employ?
S2Q112: Number of indoor staff
S2Q113: How many guards do you employ?
S2Q114: Number of guards
S2Q115: How many farmhands / gardeners do you employ?
S2Q116: Number of farmhands
S2Q12: Number of staff employed
S2Q121: How many staff are present today?
S2Q122: Number of staff present
S2Q13: Do any other non-family members (eg. friends, tenants) live in the household?
S2Q131: How many non-family members live in the house?
S2Q132: How many of them are present today?
S2Q133: Number of other adults present today
S2Q14: Total number of people present in the household today

S2Q15: The total number of people present in the household today is ${s2q14_sum_pres}. Is this correct?
S3Q0: The next section of the questionnaire relates to your house and land
S3Q1: Which of these best describes the housing type?
S3Q2: Do you own the house / apartment?
S3Q3: Do you own the land on which the house is built?
S3Q4: Was the house...?
S3Q5: How many hectares of land do you have at this location?
S3Q4: Which of these best describes your situation?
S3Q5: How much rent do you pay per month?
S3Q6: Do you own and/or rent any land elsewhere?
S3Q7: Land owned or rented
S3Q8: Current land status
S3Q9: Where is the land that you ${s3q91_land_curr}?
S3Q91: Location of land
S3Q10: Current land location
S3Q11: Location of land
S3Q91: What is the name of sublocation / region where your land is located?
S3Q7: How many hectares of land do you ${s3q91_land_curr} ${s3q111_land_loc_curr} Nairobi?
S3Q8: How many hectares of the land that you ${s3q91_land_curr} ${s3q111_land_loc_curr} Nairobi is used for agriculture?
S3Q9: Do you keep any livestock on the land that you ${s3q91_land_curr} ${s3q111_land_loc_curr} Nairobi?
S3Q10: Which of these livestock do you keep on the land that you ${s3q91_land_curr} ${s3q111_land_loc_curr} Nairobi?
S3Q8: How many rooms in your house?
S3Q114: How many bedrooms in your house?
S3Q10: How many rooms do you rent out?
S3Q11: What is the floor made from?
S3Q12: What are the walls made from?
S3Q13: What is the roof made from?
S3Q15: Transport
S3Q15: Does anyone in the household own a car or truck?
S3Q16: Does anyone in the household own a motorbike or scooter?
S3Q171: Does anyone in the household own a bicycle?
S3Q172: Does anyone in the household own an animal-drawn cart?
S3Q17: Electrical items
S3Q171: Does the household have electricity?
S3Q172: Does the household have a solar panel?
S3Q172: Do you own a refrigerator?
S3Q173: Do you own a television?
S3Q183: Do you own a radio?
S3Q183: Personal belongings
S3Q184: Does anyone in the household own a watch?

S3Q185: Does anyone in the household own a mobile phone?
S3Q191: Where does your household do most of its cooking?
S3Q192: Which sources of cooking fuel do you use?
S3Q25: What is your main source of lighting?
S3Q26: What is your main source of drinking water?
S3Q27: What is your main source of non-drinking water?
S3Q27: Do you have a water tank?
S3Q28: Do you use any water treatments for drinking water?
S3Q29: How would you rate the water quality?
S3Q30: Is the water supply always regular?
S3Q31: What type of toilet facility does your family use?
S3Q32: Where is the facility located?
S3Q33: Do you share this facility with other families?
S3Q34: What happens to waste water?
S3Q35: How do you dispose of household waste?
S3Q36: What do you do with animal waste?
S4Q11: Do you see any of the following types of wild animal around your property?
S4Q12: Which of the following carnivores have you seen around your property?
S4Q13: Which of the following primates have you seen around your property?
S4Q20: Wildlife encounters
S4Q21: Current wildlife type
S4Q22: Where do you see ${s4q21_curr_wl}s?
S4Q23: Do you ever see ${s4q21_curr_wl}s inside the house?
S4Q24: Do you ever see ${s4q21_curr_wl}s inside the kitchen?
S4Q25: Approximately how many ${s4q21_curr_wl}s do you see inside the house and/or kitchen?
S4Q26: per...
S4Q27: Do you ever see ${s4q21_curr_wl}s inside the animal housing?
S4Q28: Approximately how many ${s4q21_curr_wl}s do you see in the animal housing?
S4Q29: per...
S4Q210: Approximately how many ${s4q21_curr_wl}s do you see outside?
S4Q211: per...
S4Q30: How often do you see ${s4q21_curr_wl}s?
S4Q31: Do you think ${s4q21_curr_wl}s cause your family any of the following problems?
S4Q32: Do you think ${s4q21_curr_wl}s cause your family any of the following problems?
S4Q33: Are there any measures you use to try and control ${s4q21_curr_wl}s?
S4Q34: What type of poison do you use?
S4Q35: How many ${s4q21_curr_wl}s do you trap?
S4Q36: Number of ${s4q21_curr_wl}s trapped...
S4Q37: per...
S4Q9: Has anyone in the household ever been bitten by a ${s4q21_curr_wl}?
S5Q1: You have finished the questionnaire! Thank you very much.

S5Q2: If any absent family members have provided consent and faecal samples, collect them now
S5Q20: Household head absent - faecal sample left
S5Q21: You now need to make one swab from ${s2q1_hh_head_id}'s stool sample and place in Amies transport media. Make a second swab and place in Trizol
S5Q22: Label and scan the faecal sample pot for ${s2q1_hh_head_id}
S5Q22B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q23: Label and scan the Amies faecal swab ${s2q1_hh_head_id}
S5Q23B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q24: Label and scan the Trizol faecal swab for ${s2q1_hh_head_id}
S5Q24B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q30: Adult family members absent today
S5Q31: Current adult family member absent today
S5Q32: Current adult family member sample provided?
S5Q33: Current adult family member consent provided?
S5Q34: You now need to make one swab from ${s5q31_adlt_abst_no}'s stool sample and place in Amies transport media. Make a second swab and place in Trizol
S5Q35: Label and scan the faecal sample pot for ${s5q31_adlt_abst_no}
S5Q35B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q36: Label and scan the faecal swab for ${s5q31_adlt_abst_no}
S5Q36B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q37: Label and scan the Trizol faecal swab for ${s5q31_adlt_abst_no}
S5Q37B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q40: Child family members absent today
S5Q41: Current child family member absent today
S5Q42: Current child family member sample provided?
S5Q43: Current child family member consent provided?
S5Q44: You now need to make one swab from ${s5q41_child_abst_no}'s stool sample and place in Amies transport media. Make a second swab and place in Trizol
S5Q45: Label and scan the faecal sample pot for ${s5q41_child_abst_no}
S5Q45B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q46: Label and scan the faecal swab for ${s5q41_child_abst_no}
S5Q46B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q47: Label and scan the Trizol faecal swab for ${s5q41_child_abst_no}
S5Q47B: The barcode has not scanned correctly. Please go back and try again or enter manually
S5Q51: Capture the GPS of the current location.
S5Q52: Confirm the GPS co-ordinates

Appendix Table G2. Livestock-keeping questionnaire formatted for use with ODK Open Data Kit (ODK) Collect

label
SoQ1: Start time
SoQ3: Date
SoQ4: Device ID
SoQ5: Simcard serial number
SoQ6: Device phone number
SoQ7: Recorder Name
SoQ8: Scan household unique barcode on Household Master Sheet
SoQ8.1: The barcode has not scanned correctly. Please go back and try again or enter manually
S1Q1.0: Which types of animals are present in the households today?
S4Q1.1: Which of the following ruminant species are present in this household today?
S4Q1.2: Which of the following monogastric species are present in this household today?
S4Q1.4: Which of the following types of bird are present in this household today?
S4Q1.5: Which of the following types of bird are present in this household today?
S4Q1.6: Which of the following other species are present in this household today?
S4Q1.61: Specify other type(s) of poultry
S4Q1.3: Which of the following other species are present in this household today?
S41.31: Specify other type(s) of animals
S1Q2.0: Use a combination of the farmer's knowledge and your own observations when completing this section
S1Q2.1: Which breed(s) of beef cattle do you own?
S1Q2.2: Number of beef cattle owned
S1Q2.3: How many breeding beef bulls do you own?
S1Q2.4: How many breeding beef cows do you own?
S1Q2.5: How many beef bullocks / steers / heifers do you own?
S1Q2.6: How many beef calves (<6 months old) do you own?
S1Q2.7: Total number of beef animals
S1Q3.1: Which breed(s) of dairy cattle do you own?
S1Q3.2: Number of dairy cattle owned
S1Q3.3: How many breeding dairy bulls do you own?
S1Q3.4: How many lactating dairy cows do you own?
S1Q3.5: How many dry cows do you own?
S1Q3.6: How many heifers do you own?
S1Q3.7: How many dairy calves (<6 months old) do you own?
S1Q3.8: Total number of dairy animals
S1Q4.1: Do you have any dairy breed bullocks / steers that you are rearing for meat?
S1Q4.2: How many dairy bullocks / steers do you own?
S1Q4.3: Total number of dairybeef animals
S1Q4.4: Total number of cattle
S1Q4.5: The total number of cattle present on the premises today is ${s1q4.4_ctl_total}
S1Q4.6: How many separate groups are these animals kept in?
S1Q5.1: Specify breed of meat goat, if known
S1Q5.2: Number of meat goats owned
S1Q5.3: How many breeding bucks do you own?
S1Q5.4: How many breeding does do you own?
S1Q5.5: How many rearing stock (approx 4-12 months old) do you own?
S1Q5.6: How many kids (≤ 3 months old) do you own?
S1:
S1Q5.7: Total number of meat goats
S1Q6.1: Which breed(s) of dairy goats do you own?
S1Q6.2: Number of dairy goats owned
S1Q6.3: How many breeding dairy bucks do you own?
S1Q6.4: How many lactating dairy goats do you own?
S1Q6.5: How many dry/ pregnant goats do you own?
S1Q6.6: How many doelings (not yet kidded) do you own?
S1Q6.7: How many dairy kids(≤ 3 months old) do you own?
S1:
S1Q6.8: Total number of dairy goats
S1Q7.1: Do you have any dairy breed bucks/wethers that you are rearing for meat?
S1Q7.2: How many dairy goats being reared for meat do you own?
S1Q7.3: Total number of dairymeat goats
S1Q7.4: Total number of goats
S1Q7.5: The total number of goats present on the premises today is ${s1q7.4_gt_total}
S1Q7.6: How many separate groups are these animals kept in?
S1Q8.1: Which breed(s) of sheep do you own?
S1Q8.2: Number of sheep owned
S1Q8.3: How many breeding rams do you own?
S1Q8.4: How many breeding ewes do you own?
S1Q8.5: How many fattening lambs (4-12 months old) do you own?
S1Q8.6: How many lambs(≤ 3 months old) do you own?
S1:
S1Q8.7: Total number of sheep
S1Q8.8: The total number of sheep present on the premises today is ${s1q8.7_shp_total}
S1Q8.9: How many separate groups are these animals kept in?
S1Q9.1: Which breed(s) of pig do you own?
S1Q9.2: Number of pigs owned
S1Q9.3: How many breeding boars do you own?
S1Q9.4: How many breeding sows do you own?
S1Q9.5: How many weaners / rearers / finishers do you own (5 weeks old and over) ?
S1Q9.6: How many piglets(<5 weeks old) do you own?
S1:

S1Q9.7: Total number of pigs
S1Q9.8: The total number of pigs present on the premises today is ${s1q9.7_pig_total}
S1Q9.9: How many separate groups are these animals kept in?
S1Q10.1: Which breed(s) of rabbit do you own?
S1Q10.2: Number of rabbits owned
S1Q10.3: How many breeding bucks do you own?
S1Q10.4: How many breeding does do you own?
S1Q10.5: How many rabbit kittens / young stock do you own?
S1:
S1Q10.6: Total number of rabbits
S1Q10.7: The total number of rabbits present on the premises today is ${s1q10.6_rbt_total}
S1Q10.8: How many separate groups are these animals kept in?
S1Q11.0: Chicken Type
S1Q11.1: Current Chicken Type
S1Q11.2: Number of ${s1q11.1_curr_chkn} chickens owned
S1Q11.3: How many adult cocks do you own?
S1Q11.4: How many laying / breeding hens do you own?
S1Q11.5: How many pullets do you own (6 weeks to point of lay) ?
S1Q11.6: How many broiler chickens do you own?
S1Q11.7: How many chicks or other immature birds do you own?
S1Q11.8: How many separate groups are chickens kept in?
S1Q12.0: Other poultry Type
S1Q12.1: Current Type (Other poultry species)
S1Q12.11: Current livestock type
S1Q12.2: Number of ${s1q12.11_curr_sp} owned
S1Q12.3: How many adult males do you own?
S1Q12.4: How many laying / breeding females do you own?
S1Q12.5: How many immature birds do you own?
S1:
S1Q12.6: How many separate groups are ${s1q12.11_curr_sp} kept in?
S1:
S1Q13: How many horses / donkeys / mules do you own?
S1Q14: How many camels do you own?
S1Q15: How many cats do you own?
S1Q16: How many dogs do you own?
S1Q17: How many ${s41.31_other_spp_own} do you own?
S2Q10.2: Livestock management
S2Q10.3: Current ruminant type
S2Q10.4: Is there purpose-built housing for the ${s2q1.1_curr_rum}?
S2Q10.5: Cleaning practices
S2: How often do you clean the housing?
S2Q10.6: Do you use disinfectant to clean the housing?
S2Q13: Are the ${s2q1.1_curr_rum} allowed to graze / scavenge?
S2Q13: Which of the following do you feed the ${s2q1.1_curr_rum}?
S2Q14: Ruminant access to house
S2Q1.2: Do your ${s2q1.1_curr_rum} share or have access to your general living areas?

S2Q1.4: Do your ${s2q1.1_curr_rum} have access to food preparation areas?
S2Q11.3: Livestock management
S2Q11.4: Current monogastric type
S2Q11.5: Is there purpose-built housing for the ${s2q2.1_curr_mono}?
S2Q11.6: Cleaning practices
S2Q11.7: How often do you clean the housing?
S2: Do you use disinfectant to clean the housing?
S2Q1.7: Are the ${s2q2.1_curr_mono} allowed to graze / scavenge?
S2Q1.7: Which of the following do you feed the ${s2q2.1_curr_mono}?
S2Q1.8: Monogastrics access to house
S2Q2.2: Do your ${s2q2.1_curr_mono} share or have access to your general living areas?
S2Q2.4: Do your ${s2q2.1_curr_mono} have access to food preparation areas?
S2Q1.1: Chicken management
S2Q1.2: Current chicken type
S2Q1.3: Is there purpose-built housing for the ${s2q3.1_curr_chkn} chickens?
S2Q1.4: Chicken cleaning practices
S2Q1.5: How often do you clean the housing?
S2Q1.6: Do you use disinfectant to clean the housing?
S2Q1.7: Are the ${s2q3.1_curr_chkn} chickens allowed to scavenge?
S2: Which of the following do you feed the ${s2q3.1_curr_chkn} chickens?
S2Q2.8: Chickens access to house
S2Q3.1: Do your ${s2q3.1_curr_chkn} chickens share or have access to your general living areas?
S2Q3.3: Do your ${s2q3.1_curr_chkn} have access to food preparation areas?
S2: Poultry management
S2Q4.1: Current poultry type
S2Q2.0: Current poultry type
S2Q2.1: Is there purpose-built housing for the ${s2q4.11_curr_pltry}?
S2Q2.2: Poultry cleaning practices
S2Q2.3: How often do you clean the housing?
S2Q2.4: Do you use disinfectant to clean the housing?
S2Q2.6: Are the ${s2q4.11_curr_pltry} allowed to scavenge?
S2Q2.10: Which of the following do you feed the ${s2q4.11_curr_pltry}?
S2Q2.8: Poultry access to house
S2Q4.11: Do your ${s2q4.11_curr_pltry} share or have access to your general living areas?
S2Q4.3: Do your ${s2q4.11_curr_pltry} have access to food preparation areas?
S3Q3.5: Ruminant value chains
S3: Current ruminant type
S3Q3.6: How many different sources do your ${s3q1.1_curr_rum} come from?
S3Q3.7: Which is the main source of your ${s3q1.1_curr_rum}?
S3Q3.8: Can you tell us the location of the ${s3q1.3_source}?
S3Q3.9: Secondary sources

S3: What is the next source of your ${s3q1.1_curr_rum}?
S3Q4.0: Can you tell us the location of the ${s3q1.6_source}?
S3Q1.8: What do you use your ${s3q1.1_curr_rum} for?
S3Q1.9: What happens to breeding / milking animals at the end of their productive life?
S3Q2.4: Approximately how many ${s3q1.1_curr_rum} have you sold in the past year?
S3Q2.5: Where do you sell your ${s3q1.1_curr_rum}?
S3Q2.6: Which market / abbattoir do you use?
S3: Where do you sell milk from your ${s3q1.1_curr_rum}
S3Q4.3: Monogastric value chains
S3Q4.4: Current monogastric type
S3Q4.5: How many different sources do your ${s3q2.1_curr_mono} come from?
S3: Which is the main source of your ${s3q2.1_curr_mono}?
S3Q4.7: Can you tell us the location of the ${s3q2.3_source}?
S3Q2.8: Secondary sources
S3Q4.8: What is the next source of your ${s3q2.1_curr_mono}?
S3: Can you tell us the location of the ${s3q2.6_source}?
S3Q2.8: What do you use your ${s3q2.1_curr_mono} for?
S3Q2.9: What happens to breeding animals at the end of their productive life?
S3Q2.10: Approximately how many ${s3q2.1_curr_mono} have you sold in the past year?
S3Q2.11: Where do you sell your ${s3q2.1_curr_mono}?
S3Q2.12: Which market / abbattoir do you use?
S3Q2.13: In which subloaction is the community slab that you sell to?
S3Q3.0: Chicken value chains
S3Q3.1: Current chicken type
S3Q3.2: How many different sources do your ${s3q3.1_curr_chkn} chickens come from?
S3Q3.3: Which is the main source of your ${s3q3.1_curr_chkn} chickens?
S3Q3.4: Can you tell us the location of the ${s3q3.3_source}?
S3Q3.5: Which commercial hatchery do your chickens come from?
S3Q3.6: Secondary sources
S3Q3.7: What is the next source of your ${s3q3.1_curr_chkn} chickens?
S3Q3.8: Can you tell us the location of the ${s3q3.7_source}?
S3Q3.9: Which commercial hatchery do your chickens come from?
S3Q3.10: What do you use your ${s3q3.1_curr_chkn} chickens for?
S3Q3.11: What happens to laying birds at the end of their productive life?
S3Q3.12: Approximately how many ${s3q3.1_curr_chkn} chickens have you sold in the past year?

S3Q3.13: How do you sell your ${s3q3.1_curr_chkn} chickens?
S3Q3.14: Where do you sell your ${s3q3.1_curr_chkn} chickens?
S3Q3.15: Where do you sell carcasses from your ${s3q3.1_curr_chkn} chickens?
S3Q3.16: Where do you sell heads and feet from your ${s3q3.1_curr_chkn} chickens?
S3Q3.17: Where do you sell intestines from your ${s3q3.1_curr_chkn} chickens?
S3Q3.18: If selling to a broker:
S3Q3.19: Do you know where the broker/trader sells your ${s3q3.1_curr_chkn} chickens?
S3Q3.20: Specify:
S3Q3.15: Where do you sell eggs from your ${s3q3.1_curr_chkn} chickens
S3Q4.0: Poultry value chains
S3Q4.1: Current poultry type
S3Q4.11: Current poultry type
S3Q4.2: How many different sources do your ${s3q4.11_curr_pltry} come from?
S3Q4.3: Which is the main source of your ${s3q4.11_curr_pltry}?
S3Q4.4: Can you tell us the location of the ${s3q4.3_source}?
S3Q4.5: Secondary sources
S3Q4.6: What is the next source of your ${s3q4.11_curr_pltry}?
S3Q4.7: Can you tell us the location of the ${s3q4.6_source}?
S3Q4.8: What do you use your ${s3q4.11_curr_pltry} for?
S3Q4.9: What happens to laying birds at the end of their productive life?
S3Q4.10: Approximately how many ${s3q4.11_curr_pltry} have you sold in the past year?
S3Q4.11: Where do you sell your ${s3q4.11_curr_pltry} ?
S3Q4.13: Where do you sell eggs from your ${s3q4.11_curr_pltry}
:
S3Q16: Are there any other reasons that you keep livestock?
S4Q1: Have any of your animals had any health problems in the last six months?
S4Q2.0: Animals with health problems
S4Q2.1: Current livestock type
S4Q2.2: Have your ${s4q2.1_curr_sp} had any health problems in the last 6 months?
S4Q2.3: Did you use any antibiotics to treat the ${s4q2.1_curr_sp}?
S4Q2.4: What drug formulation(s) did you use?
S4Q2.41: Please specify "other" formulation
S4Q2.5: Antibiotic use
S4Q2.6: Current formulation
S4Q2.71: Which ${s4q2.6_curr_form} antibiotics did you use?
S4Q2.72: Which ${s4q2.6_curr_form} antibiotics did you use?
S4Q2.73: Which ${s4q2.6_curr_form} antibiotics did you use?
S4Q2.74: Which ${s4q2.6_curr_form} antibiotics did you use?

S4Q2.75: Which ${s4q2.6_curr_form} antibiotics did you use?
S4Q2.8: What was the name of the drug?
S4Q2.9: Was the ${s4q2.6_curr_form} antibiotic a...?
S4Q2.10: Do you know the dose (mg) or volume (ml) of the ${s4q2.6_curr_form} antibiotic given?
S4Q2.11: What was the amount of ${s4q2.6_curr_form} antibiotic given (per dose)?
S4Q2.12: Select whether dosage reported is in mg or ml.
S4Q2.12B: In what volume of water (litres) was this given?
S4Q2.13: How many ${s4q2.1_curr_sp} were treated?
S4Q2.14: How often was the ${s4q2.6_curr_form} antibiotic given?
S4Q2.15: For how many days was the ${s4q2.6_curr_form} antibiotic used?
S4Q2.16: Were any doses missed?
S4Q2.17: Do you know when the last dose of drug was given ?
S4Q2.18: Any other relevant information
S4Q2.19: Do you give your ${s4q2.1_curr_sp} any drugs routinely to prevent disease?
S4Q2.20: Which type(s) of drugs do you routinely use in your ${s4q2.1_curr_sp}?
S4Q2.21: Prophylactic drug use
S4Q2.22: Current drug type
S4Q2.23: Can you tell us the name of the ${s4q2.22_curr_drug} that you normally use in your ${s4q2.1_curr_sp}?
S4:
S4:
S4Q3.0: Animals with health problems
S4Q3.1: Current livestock type
S4Q3.2: Have your ${s4q3.1_curr_sp} had any health problems in the last 6 months?
S4Q3.3: Did you use any antibiotics to treat the ${s4q3.1_curr_sp}?
S4Q3.4: What drug formulation(s) did you use?
S4Q3.41: Please specify "other" formulation
S4Q3.5: Antibiotic use
S4Q3.6: Current formulation
S4Q3.71: Which ${s4q3.6_curr_form} antibiotics did you use?
S4Q3.73: Which ${s4q3.6_curr_form} antibiotics did you use?
S4Q3.74: Which ${s4q3.6_curr_form} antibiotics did you use?
S4Q3.75: Which ${s4q3.6_curr_form} antibiotics did you use?
S4Q3.8: What was the name of the drug?
S4Q3.9: Was the ${s4q3.6_curr_form} antibiotic a...?
S4Q3.10: Do you know the dose (mg) or volume (ml) of the ${s4q3.6_curr_form} antibiotic given?
S4Q3.11: What was the amount of ${s4q3.6_curr_form} antibiotic given (per dose)?
S4Q3.12: Select whether dosage reported is in mg or ml.
S4Q3.12B: In what volume of water (litres) was this given?
S4Q3.13: How many ${s4q3.1_curr_sp} were treated?

S4Q3.14: How often was the ${s4q3.6_curr_form} antibiotic given?
S4Q3.15: For how many days was the ${s4q3.6_curr_form} antibiotic used?
S4Q3.16: Were any doses missed?
S4Q3.17: Do you know when the last dose of drug was given ?
S4Q3.18: Any other relevant information
S4Q3.19: Do you give your ${s4q3.1_curr_sp} any drugs routinely to prevent disease?
S4Q3.20: Which type(s) of drugs do you routinely use in your ${s4q3.1_curr_sp}?
S4Q3.21: Prophylactic drug use
S4Q3.22: Current drug type
S4Q3.23: What ${s4q3.22_curr_drug} formulation(s) do you use?
S4Q3.24: Antibiotic use
S4Q3.25: Which in-feed antibiotic do you use?
S4Q3.26: Which in-feed anticoccidial do you use?
S4Q3.27: What is the name of the drug?
S4Q3.28: How is the in-feed ${s4q3.22_curr_drug} delivered?
S4Q3.29: Is the in-feed ${s4q3.22_curr_drug} given...?
S4Q3.30: Any other relevant information
S4Q3.31: Antibiotic use
S4Q3.32: Which in-water antibiotic do you use?
S4Q3.33: Which in-water anticoccidial do you use?
S4Q3.34: What is the name of the drug?
S4Q3.35: Is the in-water ${s4q3.22_curr_drug} given...?
S4Q3.36: How often do you replace the in-water ${s4q3.22_curr_drug}?
S4Q3.37: Any other relevant information
S4Q3.38: Can you tell us the name of the ${s4q3.22_curr_drug} that you normally use in your ${s4q3.1_curr_sp}?
S4Q4.0: Animals with health problems
S4Q4.1: Current livestock type
S4Q4.2: Have your ${s4q4.1_curr_sp} chickens had any health problems in the last 6 months?
S4Q4.3: Did you use any antibiotics to treat the ${s4q4.1_curr_sp} chickens?
S4Q4.4: What drug formulation(s) did you use?
S4Q4.41: Please specify "other" formulation
S4Q4.5: Antibiotic use
S4Q4.6: Current formulation
S4Q4.71: Which ${s4q4.6_curr_form} antibiotics did you use?
S4Q4.73: Which ${s4q4.6_curr_form} antibiotics did you use?
S4Q4.74: Which ${s4q4.6_curr_form} antibiotics did you use?
S4Q4.75: Which ${s4q4.6_curr_form} antibiotics did you use?
S4Q4.8: What was the name of the drug?
S4Q4.9: Was the ${s4q4.6_curr_form} antibiotic a...?
S4Q4.10: Do you know the dose (mg) or volume (ml) of the ${s4q4.6_curr_form} antibiotic given?
S4Q4.11: What was the amount of ${s4q4.6_curr_form} antibiotic given (per dose)?
S4Q4.12: Select whether dosage reported is in mg or ml.

S4Q4.12B: In what volume of water (litres) was this given?
S4Q4.13: How many ${s4q4.1_curr_sp} were treated?
S4Q4.14: How often was the ${s4q4.6_curr_form} antibiotic given?
S4Q4.15: For how many days was the ${s4q4.6_curr_form} antibiotic used?
S4Q4.16: Were any doses missed?
S4Q4.17: Do you know when the last dose of drug was given ?
S4Q4.18: Any other relevant information
S4Q4.19: Do you give your ${s4q4.1_curr_sp} chickens any drugs routinely to prevent disease?
S4Q4.20: Which type(s) of drugs do you routinely use in your ${s4q4.1_curr_sp} chickens?
S4Q4.21: Prophylactic drug use
S4Q4.22: Current drug type
S4Q4.23: What ${s4q4.22_curr_drug} formulation(s) do you use?
S4Q4.24: Antibiotic use
S4Q4.26: Which in-feed antibiotic do you use?
S4Q4.27: Which in-feed anticoccidial do you use?
S4Q4.28: What is the name of the drug?
S4Q4.29: How is the in-feed ${s4q4.22_curr_drug} delivered?
S4Q4.30: Is the in-feed ${s4q4.22_curr_drug} given...?
S4Q4.32: Any other relevant information
S4Q4.33: Antibiotic use
S4Q4.34: Which in-water antibiotic do you use?
S4Q4.35: Which in-water anticoccidial do you use?
S4Q4.36: What is the name of the drug?
S4Q4.37: Is the in-water ${s4q4.22_curr_drug} given...?
S4Q4.38: How often do you replace the in-water ${s4q4.22_curr_drug}?
S4Q4.39: Any other relevant information
S4Q4.40: Can you tell us the name of the ${s4q4.22_curr_drug} that you normally use in your ${s4q4.1_curr_sp} chickens?
S4Q5.0: Animals with health problems
S4Q5.1: Current livestock type
S4Q5.11: Current livestock type
S4Q5.2: Have your ${s4q5.11_curr_sp} had any health problems in the last 6 months?
S4Q5.3: Did you use any antibiotics to treat the ${s4q5.11_curr_sp}?
S4Q5.4: What drug formulation(s) did you use?
S4Q5.41: Please specify "other" formulation
S4Q5.5: Antibiotic use
S4Q5.6: Current formulation
S4Q5.71: Which ${s4q5.6_curr_form} antibiotics did you use?
S4Q5.73: Which ${s4q5.6_curr_form} antibiotics did you use?
S4Q5.74: Which ${s4q5.6_curr_form} antibiotics did you use?
S4Q5.75: Which ${s4q5.6_curr_form} antibiotics did you use?
S4Q5.8: What was the name of the drug?
S4Q5.9: Was the ${s4q5.6_curr_form} antibiotic a...?
S4Q5.10: Do you know the dose (mg) or volume (ml) of the ${s4q5.6_curr_form} antibiotic given?

S4Q5.11: What was the amount of ${s4q5.6_curr_form} antibiotic given (per dose)?
S4Q5.12: Select whether dosage reported is in mg or ml.
S4Q5.12B: In what volume of water (litres) was this given?
S4Q5.13: How many ${s4q5.11_curr_sp} were treated?
S4Q5.14: How often was the ${s4q5.6_curr_form} antibiotic given?
S4Q5.15: For how many days was the ${s4q5.6_curr_form} antibiotic used?
S4Q5.16: Were any doses missed?
S4Q5.17: Do you know when the last dose of drug was given ?
S4Q5.18: Any other relevant information
S4Q5.19: Do you give your ${s4q5.11_curr_sp} any drugs routinely to prevent disease?
S4Q5.20: Which type(s) of drugs do you routinely use in your ${s4q5.11_curr_sp}?
S4Q5.21: Prophylactic drug use
S4Q5.22: Current drug type
S4Q5.23: What ${s4q5.22_curr_drug} formulation(s) do you use?
S4Q5.24: Antibiotic use
S4Q5.25: Which in-feed antibiotic do you use?
S4Q5.26: Which in-feed anticoccidial do you use?
S4Q5.27: What is the name of the drug?
S4Q5.28: How is the in-feed ${s4q5.22_curr_drug} delivered?
S4Q5.29: Is the in-feed ${s4q5.22_curr_drug} given...?
S4Q5.30: Any other relevant information
S4Q5.31: Antibiotic use
S4Q5.32: Which in-water antibiotic do you use?
S4Q5.33: Which in-water anticoccidial do you use?
S4Q5.34: What is the name of the drug?
S4Q5.35: Is the in-water ${s4q5.22_curr_drug} given...?
S4Q5.36: How often do you replace the in-water ${s4q5.22_curr_drug}?
S4Q5.37: Any other relevant information
S4Q5.38: Can you tell us the name of the ${s4q5.22_curr_drug} that you normally use in your ${s4q5.11_curr_sp}?
S4Q6.0: Animals with health problems
S4Q6.1: Current livestock type
S4Q6.11: Current livestock type
S4Q6.2: Have your ${s4q6.11_curr_sp} had any health problems in the last 6 months?
S4Q6.3: Did you use any antibiotics to treat the ${s4q6.11_curr_sp}?
S4Q6.4: What drug formulation(s) did you use?
S4Q6.41: Please specify "other" formulation
S4Q6.5: Antibiotic use
S4Q6.6: Current formulation
S4Q6.71: Which ${s4q6.6_curr_form} antibiotics did you use?
S4Q6.73: Which ${s4q6.6_curr_form} antibiotics did you use?
S4Q6.74: Which ${s4q6.6_curr_form} antibiotics did you use?
S4Q6.75: Which ${s4q6.6_curr_form} antibiotics did you use?
S4Q6.8: What was the name of the drug?

S4Q6.9: Was the ${s4q6.6_curr_form} antibiotic a...?
S4Q6.10: Do you know the dose (mg) or volume (ml) of the ${s4q6.6_curr_form} antibiotic given?
S4Q6.11: What was the amount of ${s4q6.6_curr_form} antibiotic given (per dose)?
S4Q6.12: Select whether dosage reported is in mg or ml.
S4Q6.12B: In what volume of water (litres) was this given?
S4Q6.13: How many ${s4q6.1_curr_sp} were treated?
S4Q6.14: How often was the ${s4q6.6_curr_form} antibiotic given?
S4Q6.15: For how many days was the ${s4q6.6_curr_form} antibiotic used?
S4Q6.16: Were any doses missed?
S4Q6.17: Do you know when the last dose of drug was given ?
S4Q6.18: Any other relevant information
S4Q6.19: Do you give your ${s4q6.11_curr_sp} any drugs routinely to prevent disease?
S4Q6.20: Which type(s) of drugs do you routinely use in your ${s4q6.11_curr_sp}?
S4Q6.21: Prophylactic drug use
S4Q6.22: Current drug type
S4Q6.23: What ${s4q6.22_curr_drug} formulation(s) do you use?
S4Q6.24: Antibiotic use
S4Q6.25: Which in-feed antibiotic do you use?
S4Q6.26: Which in-feed anticoccidial do you use?
S4Q6.27: What is the name of the drug?
S4Q6.28: How is the in-feed ${s4q6.22_curr_drug} delivered?
S4Q6.29: Is the in-feed ${s4q6.22_curr_drug} given...?
S4Q6.30: Any other relevant information
S4Q6.31: Antibiotic use
S4Q6.32: Which in-water antibiotic do you use?
S4Q6.33: Which in-water anticoccidial do you use?
S4Q6.34: What is the name of the drug?
S4Q6.35: Is the in-water ${s4q6.22_curr_drug} given...?
S4Q6.36: How often do you replace the in-water ${s4q6.22_curr_drug}?
S4Q6.37: Any other relevant information
S4Q6.38: Can you tell us the name of the ${s4q6.22_curr_drug} that you normally use in your ${s4q6.1_curr_sp}?
S5Q2.19: You have finished the survey! Thank you very much for your time.
S5Q2.20: End time

Appendix Table G3. Individual human questionnaire formatted for use with ODK Open Data Kit (ODK) Collect

label
S1Q1: Start time
S1Q3: Date
S1Q4: Device ID
S1Q5: Simcard serial number
S1Q6: Device phone number
S1Q7: Recorder Name
S1Q8: Scan household unique barcode on Household Master Sheet
S1Q81: The barcode has not scanned correctly. Please go back and try again or enter manually
S1Q9: Does the respondent live on site?
S1Q10: Label and scan barcode for respondent's consent form
S1Q101: The barcode has not scanned correctly. Please go back and try again or enter manually
S2Q1: Does the respondent know their date of birth?
S2Q2: Date of birth
S2Q21: What is the respondent's date of birth?
S2Q22: Respondent's age
S2Q23: Respondent's age category
S2Q24: The respondent's age is ${s2q22_resp_age}. Is this correct?
S2Q25: Respondent's age
S2Q26: What is the respondent's age?
S2Q27: Select:
S2Q28: Respondent's age category
S2Q31: Confirm that the respondent is over 18
S2Q32: If the respondent is under 18, confirm you have the consent of a parent or guardian before proceding
S2Q4: Is the respondent:
S1Q5: Is this respondent the head of the household?
S2Q6: What is the first name of the household head?
S2Q61: Confirm the first name of the household head with the household Master Sheet
S2Q7: Which gender is the respondent?
S2Q80: Relationship to household head
S2Q81: Respondent's gender
S2Q82: Respondent's gender
S2Q83: Relationship category
S2Q84: What relation is the respondent to ${s2q6_hh_head_id}?
S2Q9: What position does the respondent hold in the household?
S2Q10: What education has the respondent had?
S2Q11: Has the respondent attended school in the last 6 months?
S2Q12: What is the respondent's occupation?
S2Q13: Does the respondent work with any of the following OUTSIDE the household?
S2Q14: Which of these activities does the respondent perform with the livestock in this household?
S2Q151: What ethnicity is the respondent?
S2Q152: Which tribe is the respondent?
S3Q11: Is the respondent the main person responsible for buying food in this household?
S3Q12: Does the respondent regularly help to prepare food in this household?
S3Q2: In a normal week, on how many days do you BUY meat to cook or eat at home?
S3Q3: Which of these meats do you buy?
S3Q4: Where and how do you buy meat?
S3Q41: Current meat type
S3Q42: Which of these forms of ${s3q41_curr_meat} do you buy?
S3Q43: Which of these forms of ${s3q41_curr_meat} do you buy?
S3Q44: Which of these forms of ${s3q41_curr_meat} do you buy?
S3Q51: Do you normally buy...
S3Q52: Do you normally buy...
S3Q6: How much ${s3q41_curr_meat} (kg) in any form do you buy in a typical week?
S3Q71: Where do you buy ${s3q41_curr_meat} ?
S3Q72: Do you buy any particular brand of ${s3q41_curr_meat} ?
S3Q73: Do you buy any particular brand of ${s3q41_curr_meat} ?
S3Q74: Where do you buy ${s3q41_curr_meat} ?
S3Q75: Where do you buy ${s3q41_curr_meat} ?
S3Q81: Where do you buy ${s3q52_chckn_liv} chicken ?
S3Q82: Please specify where you buy ${s3q52_chckn_liv} chicken ?
S3Q83: Do you buy any particular brand of ${s3q52_chckn_liv} chicken ?
S3Q10: Have you bought any more meat than normal in the last two weeks (eg for festive periods)
S3Q11: Which of these meats did you additionally buy?
S3Q12: In a normal week, on how many days do you BUY milk to consume at home?
S3Q13: What type of milk do you buy?
S3Q140: Milk purchasing
S3Q141: Milk species
S3Q142: What form of ${s3q141_milk_sp_curr} milk do you buy?
S3Q143: What form of ${s3q141_milk_sp_curr} milk do you buy?
S3Q144: Where do you buy ${s3q141_milk_sp_curr} milk?
S3Q145: Do you buy any particular brand of ${s3q141_milk_sp_curr} milk?
S3Q146: Do you boil ${s3q141_milk_sp_curr} milk before it is consumed by the family?
S3Q147: Is the mala you buy made from...?
S3Q15: How often do you BUY eggs to consume at home?
S4Q16: Where do you buy eggs?
S5Q17: What type of eggs do you buy?
S6Q18: Do you usually buy any particular brand of eggs?

S7Q19: Which brand(s)?
S3Q20: Do you ever eat any livestock or livestock products that you produce yourself?
S3Q21: Which products do you consume from your own livestock?
S3Q220: Home-produced dairy
S3Q221: How do you prepare milk you produce yourself?
S3Q222: Do you make mala?
S3Q223: Do you make yoghurt?
S3Q23: Production of mala/yoghurt
S3Q231: What do you prepare mala from?
S3Q232: What do you prepare yoghurt from?
S3Q24: Which of these meats do you eat from the livestock that you produce?
S3Q250: Consumption of own meat products
S3Q251: Current meat type
S3Q252: Are the chickens that you eat yourself…?
S3Q253: Which types of poultry that you produce do you eat yourself…?
S3Q254: Where are the animals that you use to produce your own ${s3q251_curr_meat} meat normally kept?
S3Q255: Do the animals enter this household prior to slaughter?
S3Q256: Where are animals slaughtered?
S3Q257: Where are animals slaughtered?
S3Q258: Where are animals slaughtered?
S4Q0: This next section is about foods that you have consumed IN THE LAST 7 DAYS
S4Q11: Which of these food types have you consumed IN THE LAST 7 DAYS?
S4Q12: Which of these food types have you consumed IN THE LAST 7 DAYS?
S4Q2: Food frequency
S4Q3: Current food type
S4Q31: On how many days out of the last 7 did you eat ${s4q3_curr_food}?
S4Q32: Where did you eat the eggs?
S4Q2: Which of these types of meat have you consumed IN THE LAST 7 DAYS?
S4Q3: Where and how did you eat meat in the last 7 days?
S4Q31: Current meat type
S4Q32: Which of these forms of ${s4q31_curr_meat} did you eat?
S4Q33: Which of these forms of ${s4q31_curr_meat} did you eat?
S4Q34: Which of these forms of ${s4q31_curr_meat} did you eat?
S4Q35: On how many days out of the last 7 did you eat ${s4q31_curr_meat} (In any form)?
S4Q36: In which of these places did you eat ${s4q31_curr_meat} (In any form)?
S4Q37: In which of these places did you eat ${s4q31_curr_meat} (In any form)?
S4Q38: Do you know what kind of of ${s4q31_curr_meat} you ate?
S4Q4: Which of these types of dairy product have you consumed IN THE LAST 7 DAYS?
S4Q5: Where and how did you consume dairy products in the last 7 days?
S4Q61: Current dairy product type

S4Q62: On how many days out of the last 7 did you consume ${s4q61_curr_dairy}?
S4Q63: In which of these places did you consume ${s4q61_curr_dairy}?
S4Q64: How was the ${s4q61_curr_dairy} you drank prepared?
S4Q7: Which of these types of fish have you consumed IN THE LAST 7 DAYS?
S4Q8: How often did you consume fish products in the last 7 days?
S4Q81: Current fish type
S4Q82: On how many days out of the last 7 did you consume ${s4q81_curr_fish}?
S4Q9: Do you ever eat meat?
S4Q90: Meats eaten more rarely
S4Q91: How often do you eat beef?
S4Q92: How often do you eat mutton or lamb?
S4Q93: How often do you eat goat?
S4Q94: How often do you eat pork (any form)?
S4Q95: How often do you eat rabbit?
S4Q96: How often do you eat chicken?
S4Q97: How often do you eat any other kind of poultry (duck, turkey, quail, etc)?
S5Q0: We are now going to ask you some questions about your health, and perform an examination
S5Q1: Has the respondent had any of the following in the last 7 days?
S5Q2: What was the appearance of the diarrhoearic stools?
S5Q21: Did the stool smell different?
S5Q22: When was the first episode of diarrhoea?
S5Q23: When was the first episode of diarrhoea?
S5Q3: Has the respondent used any of the following in the past two weeks?
S5Q31: Medicine used
S5Q32: Current medication type
S5Q33: Select which antibiotic has been used
S5Q34: What is the name of the ${s5q32_curr_med_type} medication used?
S5Q35: Do you know the dosage (mg)?
S5Q36: Do you know the frequency?
S5Q37: Has the patient missed any doses, or had any problems with compliance?
S5Q38: Any other relevant information
S5Q4: How long did the diarrhoea take to respond to medication? (days)
S5Q40: Medication failure
S5Q41: Did you have to change medication at any point due to failure of the first one?
S5Q42: Drug changed from…?
S5Q43: Drug changed to…?
S5Q5: Has the participant had any conditions requiring antibiotic treatment in the last 6 months?
S5Q50: Antibiotic use
S5Q51: What was the antibiotic prescription related to?
S5Q52: Select which antibiotic has been used
S5Q53: What is the name of the antibiotic used?

S4Q54: Do you know when the last dose of drug was given ?
S4Q55: Do you know the dose (mg) antibiotic given?
S4Q56: What was the amount of antibiotic given (per dose)?
S4Q57: How often was the antibiotic given?
S4Q58: For how many days was the antibiotic used?
S4Q59: Were any doses missed?
S5Q6: Any other relevant information
S6Q1: Appearance
S6Q2: Hydration status
S6Q3: Capillary refill
S6Q4: Pulse rate
S6Q5: Respiratory rate
S6Q6: Temperature (ºC)
S6Q7: Blood pressure
S6Q71: Blood pressure (systolic)
S6Q72: Blood pressure (diastolic)
S6Q73: blood pressure is ${s6q71_bp_sys} over ${s6q72_bp_dias}
S6Q8: Mid upper arm circumference (cm)
S6Q9: Weight (kg)
S6Q10: Height (cm)
S7Q1: Has the participant provided a stool sample?
S7Q2: Is the participant willing to have three faecal swabs taken?
S7Q21: Take three faecal swabs and place in Amies transport media
S7Q22: Is the participant willing to have two nasal swabs taken?
S7Q23: Take two nasal swabs and place one in Trizol and one in TSB transport media
S7Q3: You have finished the questionnaire! Thank you very much for your participation
S7Q40: Nasal swabs
S7Q41: Label and scan the barcode of the partcipant's TSB nasal swab
S7Q42: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q43: Label and scan the barcode of the participant's Trizol nasal swab
S7Q44: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q45: Send the nasal swab in TSB to KEMRI. Send the swab in Trizol to ILRI
S7Q5: Faecal samples (pot)
S7Q50: You now need to make one swab from the stool sample and place in Amies transport media.
S7Q51: Label and scan the barcode of the patient's stool sample pot
S7Q52: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q53: Label and scan the barcode of the patient's faecal swab
S7Q54: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q55: Send the faecal pot to KEMRI and the swab to UoN
S7Q5B: Faecal samples (no pot)

S7Q51B: Label and scan the barcode of the patient's first faecal swab
S7Q52B: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q53B: Label and scan the barcode of the patient's second faecal swab
S7Q54B: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q55B: Label and scan the barcode of the patient's third faecal swab
S7Q56B: The barcode has not scanned correctly. Please go back and try again or enter manually
S7Q57B: Send two faecal swabs to KEMRI and one swab to UoN
S7Q6: Faecal samples (pot)
S7Q60: You now need to make one swab from the stool sample and place in Trizol
S7Q61: Label and scan the barcode of the patient's Trizol pot
S7Q62: The barcode has not scanned correctly. Please go back and try again or enter manually
S8Q1: End time

Appendix Table G4. Human drug store questionnaire formatted for use with ODK Open Data Kit (ODK) Collect

label
Start time
Date
Collect the GPS coordinates of this store
Recorder Name
Respondent
What is the respondent's age?
What is the respondent's gender?
What is the respondent's highest educational level?
How many people work in this shop?
What type of business is this?
What is your role in this pharmacy?
Respondent education
For how long have you worked in this premise?
Do you have specific training in medical sciences?
Have you received specific training on appropriate use of antibiotics?
Products sold
What kind of products do you sell in your shop?
What antibiotic classes do you have in your shop now?
Formulations
What formulations are tetracylines available in?
What formulations are Sulfonamides available in?
What formulation is Sulfonamide+Trime available in?
What formulations are Penicillins available in?
What formulation is Penicillin+Streptomycin available in?
What formulation are Aminoglycosides available in?
What formulation are 1st Cephalosporins available in?
What formulation are 2nd Cephalosporins available in?
What formulation are 3rd Cephalosporins available in?
What formulation are 4th Cephalosporins available in?
What formulations are Amphenicols available in?
What formulations are Macrolides available in?
What formulations are Quinolones available in?
What formulations are Floroquinolones available in?
What formulation is Fosfomycin available in?
What formulation are Polypeptides (colistin) available in?
What formulations are Glycopeptides (avopracin) available in?
What formulations are lincosamides available in?
What formulation is Nitroimidazole available in?
What formulations are carbapenems available in?
Sales estimate dynamics
Of these antibiotic classes ${antibiotics_sold} please indicate the four most commonly prescribed/sold antibiotic class?
Sales estimate dynamics cont..
Of these antibiotic classes ${antibiotics_sold} please indicate the four least commonly commonly prescribed/sold antibiotic class?
Sales estimate
Have your antibiotic sales during the last one year increased, decreased or stayed the same compared to the previous year?
Reasons for sales change
What are the most important reasons for this change?
Antibiotic sources
What factors do you take into account when choosing a supplier?
Where do you acquire your antibiotics from?
Reasons for source choice
Of these sources ${antibiotics_purchase} what is the most important source?
Do you get regular supplies?
Reasons for sales irregular supply
What are the reasons for irregular supply?
Are there certain antibiotic classes you would like to purchase from your suppliers but you are unable?
Abtibiotics unable to buy
Which of these antibiotic classes are you not able to buy from your suppliers?
What are the reasons for inability to purchase the antibiotics?
Customer characteristics
On estimate how many customers have you had in the last one week?
On estimate how many customers have you sold antibiotics to in the last one week?
On average how many customers without a prescription do you serve in a week?
On average how many customers with a prescriptions do you serve in a week?
For customers with a written prescription do you change/substitute the antibiotics indicated ?
Prescription change
What are the reasons for the change/substitution?
What antibiotic classes are only sold to customer with a written prescription?
Customer characteristics cont..
For the antibiotic customers, what is the most common gender?

What are the common presented complaints for purchasing antibiotics?
Where do most of your customers come from?
Purchase process
Which of the following information do you give customers when buying antibiotics ?
What factors do you take into account when recommending a certain antibiotic to a customer?
How do you store your antibiotics?
Antimicrobial resistance
Have you heard of any of the following terms?
Please indicate whether you agree with the following statements
Antibiotic resistance occurs when your body becomes resistant to antibiotics and they no longer work as well
Many infections are becoming increasingly resistant to treatment by antibiotics
If bacteria are resistant to antibiotics, it can be very difficult or impossible to treat the infections they cause
Antibiotic resistance is an issue that could affect me or my family
Please indicate whether you agree with the following statements
Antibiotic resistance is an issue in other countries but not here
Antibiotic resistance is only a problem for people who take antibiotics regularly
Bacteria which are resistant to antibiotics can be spread from person to person
Antibiotic-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous
Do you agree the following actions would help address the problem of antibiotic resistance?
People should use antibiotics only when they are prescribed by a doctor or nurse
Farmers should give fewer antibiotics to food-producing animals
People should not keep antibiotics and use them later for other illnesses
Parents should make sure all of their children's vaccinations are up-to-date
Do you agree the following actions would help address the problem of antibiotic resistance?
People should wash their hands regularly
Doctors should only prescribe antibiotics when they are needed
The governments should reward the development of new antibiotics
Pharmaceutical companies should develop new antibiotics
Do you agree with following statements?
Antibiotic resistance is one of the biggest problems the world faces
Antibiotic resistance is one of the biggest problems Kenya faces

Medical experts will solve the problem of antibiotic resistance before it becomes too serious
Do you agree with following statements?
Everyone needs to take responsibility for using antibiotics responsibly
There is not much people like me can do to stop antibiotic resistance
I am worried about the impact that antibiotic resistance will have on my health, and that of my family
I am not at risk of getting an antibiotic resistant infection, as long as I take my antibiotics correctly
Licence
What proportion of the antibiotics sold are generic brands?
Do you require a licence to operate your business?
You have finished the questionnaire! Thank you very much.
Kindly tick where possible
Shop is well stocked
Shop uses an electronic till system
End time

Appendix Table G5. Veterinary drug store questionnaire formatted for use with ODK Open Data Kit (ODK) Collect

label
Start time
Date
Collect the GPS coordinates of this store
Recorder Name
Respondent
What is the respondent's age?
What is the respondent's gender?
What is the respondent's highest educational level?
What type of business is this?
How many people work in this shop?
What is your role in this pharmacy?
Respondent education
For how long have you worked in this premise?
Do you have specific training in veterinary sciences?
Have you received specific training on appropriate use of antibiotics?
Products sold
What kind of products do you sell in your shop?
What antibiotic classes do you have in your shop now?
Formulations
What formulations are tetracylines available in?
What formulations are Sulfonamides available in?
What formulation is Sulfonamide+Trime available in?
What formulations are Penicillins available in?
What formulation is Penicillin+Streptomycin available in?
What formulation are Aminoglycosides available in?
What formulation are 1st Cephalosporins available in?
What formulation are 2nd Cephalosporins available in?
What formulation are 3rd Cephalosporins available in?
What formulation are 4th Cephalosporins available in?
What formulations are Amphenicols available in?
What formulations are Macrolides available in?
What formulations are Quinolones available in?
What formulations are Floroquinolones available in?
What formulation is Fosfomycin available in?
What formulations are Polypeptides (colistin) available in?
What formulations are Glycopeptides (avopracin) available in?
What formulations are lincosamides available in?
What formulation is Nitroimidazole available in?
What formulations are carbapenems available in?
Sales estimate dynamics
Of these antibiotic classes ${antibiotics_sold} please indicate the four most commonly prescribed/sold antibiotic class?
Sales estimate dynamics cont..
Of these antibiotic classes ${antibiotics_sold} please indicate the four least commonly commonly prescribed/sold antibiotic class?
Sales estimate
Have your antibiotic sales during the last one year increased, decreased or stayed the same compared to the previous year?
Reasons for sales change
What are the most important reasons for this change?
Antibiotic sources
What factors do you take into account when choosing a supplier?
Where do you acquire your antibiotics from?
Reasons for source choice
Of these sources ${antibiotics_purchase} what is the most important source?
Do you get regular supplies?
Reasons for sales irregular supply
What are the reasons for irregular supply?
Are there certain antibiotic classes you would like to purchase from your suppliers but you are unable?
Abtibiotics unable to buy
Which of these antibiotic classes are you not able to buy from your suppliers?
What are the reasons for inability to purchase the antibiotics?
Customer characteristics
On estimate how many customers have you had in the last one week?
On estimate how many customers have you sold antibiotics to in the last one week?
Customer characteristics cont..
Of the following who are your customers of antibiotics?
Customer characteristics cont..
On average, how many veterinarians do you serve in a week?
On average, how many para veterinarians do you serve in a week?
On average, how many dairy farmers do you serve in a week?
On average, how many pig farmers do you serve in a week?
On average, how many poultry farmers do you serve in a week?
On average, how many beef farmers do you serve in a week?
On average, how many rabbit farmers do you serve in a week?
Antibiotic sales by category
Which of these antibiotic classes are most commonly sold to pig farmers?
Antibiotic sales by category
Which of these antibiotic classes are most commonly sold to dairy farmers?

Antibiotic sales by category cont..
Which of these antibiotic classes are most commonly sold to beef farmers?
Antibiotic sales by category cont..
Which of these antibiotic classes are most commonly sold to poultry farmers?
Antibiotic sales by category cont..
Which of these antibiotic classes are most commonly sold to rabbit farmers?
Purchase process
How often do farmers make a request/demand (without advice) for a specific antibiotic?
How often do farmers visit your shop with an antibiotic prescription?
How often do animal health professionals make a request/demand (without advice) for a specific antibiotic?
Where do most of your customers come from?
Purchase process cont...
Which of the following information do you give customers when buying antibiotics ?
What factors do you take into account when recommending a certain antibiotic to a customer?
How do you store your antibiotics?
Antimicrobial resistance
Have you heard of any of the following terms?
Please indicate whether you agree with the following statements
Antibiotic resistance occurs when your body becomes resistant to antibiotics and they no longer work as well
Many infections are becoming increasingly resistant to treatment by antibiotics
If bacteria are resistant to antibiotics, it can be very difficult or impossible to treat the infections they cause
Antibiotic resistance is an issue that could affect me or my family
Please indicate whether you agree with the following statements
Antibiotic resistance is an issue in other countries but not here
Antibiotic resistance is only a problem for people who take antibiotics regularly
Bacteria which are resistant to antibiotics can be spread from person to person
Antibiotic-resistant infections could make medical procedures like surgery, organ transplants and cancer treatment much more dangerous
Do you agree the following actions would help address the problem of antibiotic resistance?
People should use antibiotics only when they are prescribed by a doctor or nurse
Farmers should give fewer antibiotics to food-producing animals
People should not keep antibiotics and use them later for other illnesses

Parents should make sure all of their children's vaccinations are up-to-date
Do you agree the following actions would help address the problem of antibiotic resistance?
People should wash their hands regularly
Doctors should only prescribe antibiotics when they are needed
The governments should reward the development of new antibiotics
Pharmaceutical companies should develop new antibiotics
Do you agree with following statements?
Antibiotic resistance is one of the biggest problems the world faces
Antibiotic resistance is one of the biggest problems Kenya faces
Medical experts will solve the problem of antibiotic resistance before it becomes too serious
Do you agree with following statements?
Everyone needs to take responsibility for using antibiotics responsibly
There is not much people like me can do to stop antibiotic resistance
I am worried about the impact that antibiotic resistance will have on my health, and that of my family
I am not at risk of getting an antibiotic resistant infection, as long as I take my antibiotics correctly
Licence
What proportion of the antibiotics sold are generic brands?
Do you require a licence to operate your business?
You have finished the questionnaire! Thank you very much.
Kindly tick where possible
Shop is well stocked
Shop uses an electronic till system
End time

11.6 Appendix H: Contributions and affiliations of people involved in the UrbanZoo project.

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> **No one can whistle a symphony. It takes a whole orchestra to play it.**
>
> **Halford E. Luccock, 1885 – 1960**

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11.7 Appendix I: Related publications

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Original Articles

Are Food Animals Responsible for Transfer of Antimicrobial-Resistant *Escherichia coli* or Their Resistance Determinants to Human Populations? A Systematic Review

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Abstract

The role of farm animals in the emergence and dissemination of both AMR bacteria and their resistance determinants to humans is poorly understood and controversial. Here, we systematically reviewed the current evidence that food animals are responsible for transfer of AMR to humans. We searched PubMed, Web of Science, and EMBASE for literature published between 1940 and 2016. Our results show that eight studies (18%) suggested evidence of transmission of AMR from food animals to humans, 25 studies (56%) suggested transmission between animals and humans with no direction specified and 12 studies (26%) did not support transmission. Quality of evidence was variable among the included studies; one study (2%) used high resolution typing tools, 36 (80%) used intermediate resolution typing tools, six (13%) relied on low resolution typing tools, and two (5%) based conclusions on co-occurrence of resistance. While some studies suggested to provide evidence that transmission of AMR from food animals to humans may occur, robust conclusions on the directionality of transmission cannot be drawn due to limitations in study methodologies. Our findings highlight the need to combine high resolution genomic data analysis with systematically collected epidemiological evidence to reconstruct patterns of AMR transmission between food animals and humans.

Keywords: antimicrobial resistance, *Escherichia coli*, food animals, humans, systematic review

Introduction

The evolution of microbial pathogens that enables them to evade antimicrobial treatment has been regarded as a serious public health threat (Davies, 2011; WHO, 2015; O'Neill, 2016).

At present, the role of farm animals in the emergence and dissemination of both antimicrobial resistance (AMR) bacteria and their resistance determinants to humans is poorly understood and controversial (Marshall and Levy, 2011; Woolhouse *et al.*, 2015). Various studies have suggested that AMR bacteria and their AMR determinants can be transmitted from food animals to humans via direct contact and/or through animal products (Howells and Joynson, 1975; Aminov and Mackie, 2007; Jakobsen *et al.*, 2010; Overdevest *et al.*, 2011; Kluytmans *et al.*, 2013; Voets *et al.*, 2013). However, most of these studies have relied heavily on traditional microbiology and molecular tools, such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). These tools may not have sufficient discriminatory power to provide evidence of the transmission (or not) of resistant bacteria and their AMR determinants and, importantly, to infer the direction of the transmission (de Been *et al.*, 2014; Woolhouse *et al.*, 2015). Two key pathways of transfer of resistant bacteria

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and their AMR determinants from food animals to humans have been hypothesized: (i) horizontal transmission of AMR genes of food animal origin and (ii) clonal transfer of resistant bacteria of food animal origin to humans (Lipsitch *et al.*, 2002; Chang *et al.*, 2015). Evidence from a recent systematic review suggests that a proportion of human cephalosporin-resistant *Escherichia coli (E. coli)* clones, often associated with human disease, originate from food animals through food products (Lazarus *et al.*, 2015), though these products could have been contaminated elsewhere in the production chain (Wooldridge, 2012).

Evidence either supporting or refuting the claim that dissemination of AMR bacteria or their resistance determinants from food animals to humans is occurring will be key to the development of effective policies on antibiotic stewardship and infection control for both human and animal health. To address this knowledge gap, we performed a systematic review to (i) explore the current evidence that food animals are of the source of resistant *E. coli* and their AMR determinants in humans, (ii) examine and summarize the kinds of evidence used to support, or not support, transfer of resistant *E. coli* and their AMR determinants to humans, and (iii) make recommendations for future studies to address this question. *E. coli* is found in both human and food animal populations (Neidhardt *et al.*, 1996), and it has recently been categorized as one of the priority pathogens that pose the greatest threat to human health due to widespread AMR (WHO, 2017). It is for these reasons that, when considering transmission between hosts, we chose to focus on *E. coli*.

Methods

Data sources and search strategy

A systematic literature search according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines (Liberati *et al.*, 2009) was performed. Searches were carried out in multiple electronic databases: PubMed, Web of Science, and EMBASE for research articles published between 1940 and 2016; and Scopus for research articles published between 1960 and 2016 without geographical and language restriction. We did initial and subsequent keyword searches with various combinations of search terms: *E. coli*, AMR terminologies, human, and food animal descriptors (Supplementary Data; Supplementary Data are available online at www.liebertpub.com/fpd).

Selection criteria and data extraction

Articles were included if they comprised an original research published in a peer reviewed journal, and investigated transmission of resistant *E. coli* and/or AMR determinants between humans and food animals. Articles were excluded if (i) they reported only agents other than *E. coli*; (ii) they studied nonfood animals; (iii) they focused exclusively on food animals or humans without any overlap between the two populations and/or (iv) they focused exclusively on food of animal origin. Article searches and screening were performed by considering article titles and abstracts for inclusion according to the search criteria. Data extraction from studies was performed by one author (D.M.M.) and independently checked by another author (B.v.B.) using a customized checklist.

Data analysis

For all included studies we categorized the direction of AMR transmission according to the authors' conclusions: (i) studies suggesting to provide evidence of transmission from food animals to humans with direction specified; (ii) studies suggesting to provide evidence of transmission from humans to food animals with direction specified; (iii) studies suggesting overlap indicating the possibility of between-host AMR transmission, with no direction specified; and (iv) studies suggesting no evidence of transmission in either direction.

The quality of evidence was assessed using a customized Grading of Recommendations Assessment, Development and Evaluation (GRADE) system (Godfray *et al.*, 2013). Each article was matched to the following categories: (i) high resolution typing: studies using whole genome sequencing (WGS) and phylogenetic analysis; (ii) intermediate resolution typing: studies carrying out genetic characterisation through molecular tools such as MLST; (iii) low resolution typing: studies using tools such as PFGE; or (iv) co-occurrence of resistances: studies comparing AMR phenotypes between the two populations.

Additionally, we assessed the methodological quality of the articles included in the review by adapting a standardized quality assessment (Centre for Reviews Dissemination, 2009). Each article was evaluated based on two items aimed at assessing potential biases including study design (a ctive, passive) and spatiotemporal matching (no matching, temporal matching only, spatial matching only, and both temporal and spatial matching).

Because of heterogeneity of the studies (regarding typing tools, antibiotics investigated and quality of evidence) we did not perform a meta-analysis. However, we used Fisher's exact tests using R package ''stats'' (R Core Team, 2017) to describe associations between direction of transmission, selection bias variables and nature of transmission (clonal, determinant or both). We considered $p < 0.05$ to be statistically significant.

Results

Description of included studies

Of the 5662 distinct articles retrieved, 256 studies were reviewed (Fig. 1); and 45 studies met all inclusion criteria (Supplementary Table S1 in the Supplementary Data). The 45 studies were geographically diverse and included 20 countries, with 26 from Europe, 11 from Asia, five from North America, two from Africa, and one from the Middle East (Fig. 2).

Although the studies span five decades, there has been an increasing number of studies on this subject in recent years; with 56% of the studies published since 2010 (Supplementary Fig. S1 in the Supplementary Data). Twenty two studies (49%) had both temporal and spatial matching for human and food animal sampling, while seven (16%) had temporal matching only, and 16 (35%) were not temporally or spatially matched. We found no statistical associations between whether direction of transmission was inferred and study design or spatiotemporal matching.

Studies in our review reported different livestock species, either alone or in combination with other species. Of the eight studies that suggested transfer of AMR from food animals to humans, seven studies were based on poultry isolates and one study on pig isolates (Supplementary Fig. S2 in the

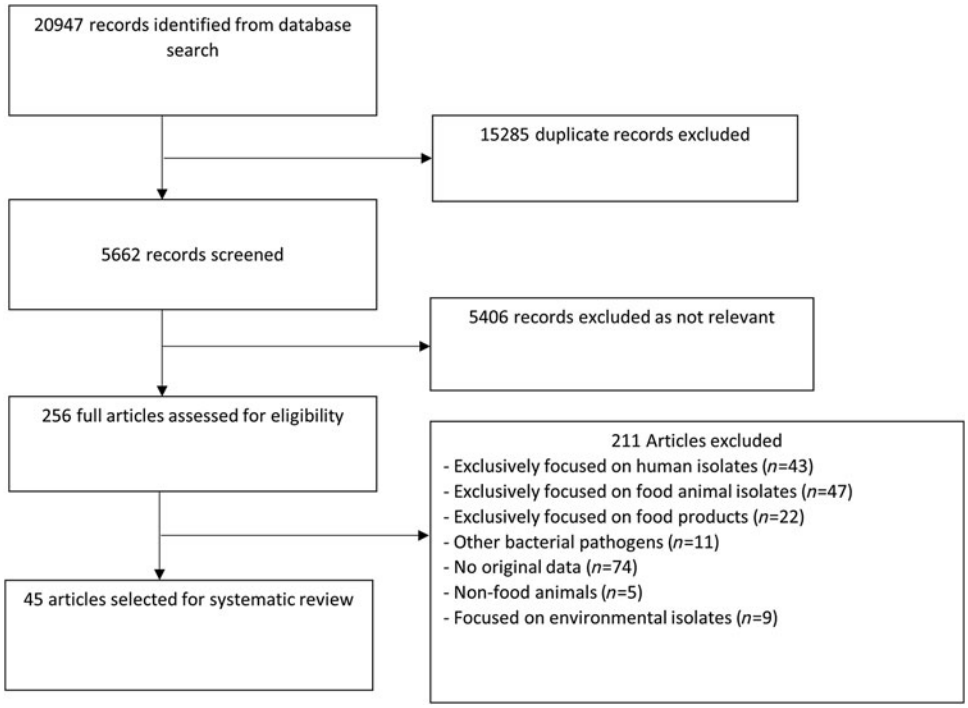

FIG. 1. Flow diagram showing the selection of studies for inclusion.

Supplementary Data). Among the studies, 13 antibiotic classes were reported, either alone or in combination with other classes (Supplementary Fig. S3 in the Supplementary Data).

Overall, eight studies (18%) suggested to have data to support transfer of AMR bacteria and/or their AMR determinants from food animals to humans (Levy, 1978; Al-Ghamdi *et al.*, 1999; van den Bogaard *et al.*, 2001; Hammerum *et al.*, 2006; Johnson *et al.*, 2006; Leverstein-van Hall *et al.*, 2011; Giufre *et al.*, 2012; Dierikx *et al.*, 2013), while 25 studies (56%) presented data showing overlap of AMR bacteria and AMR determinants between food animals and humans, indicating the possibility of between-host AMR transmission but with no direction specified (Jorgensen, 1983; Oppegaard *et al.*, 2001; Winokur *et al.*, 2001; Ho *et al.*, 2009, 2010;

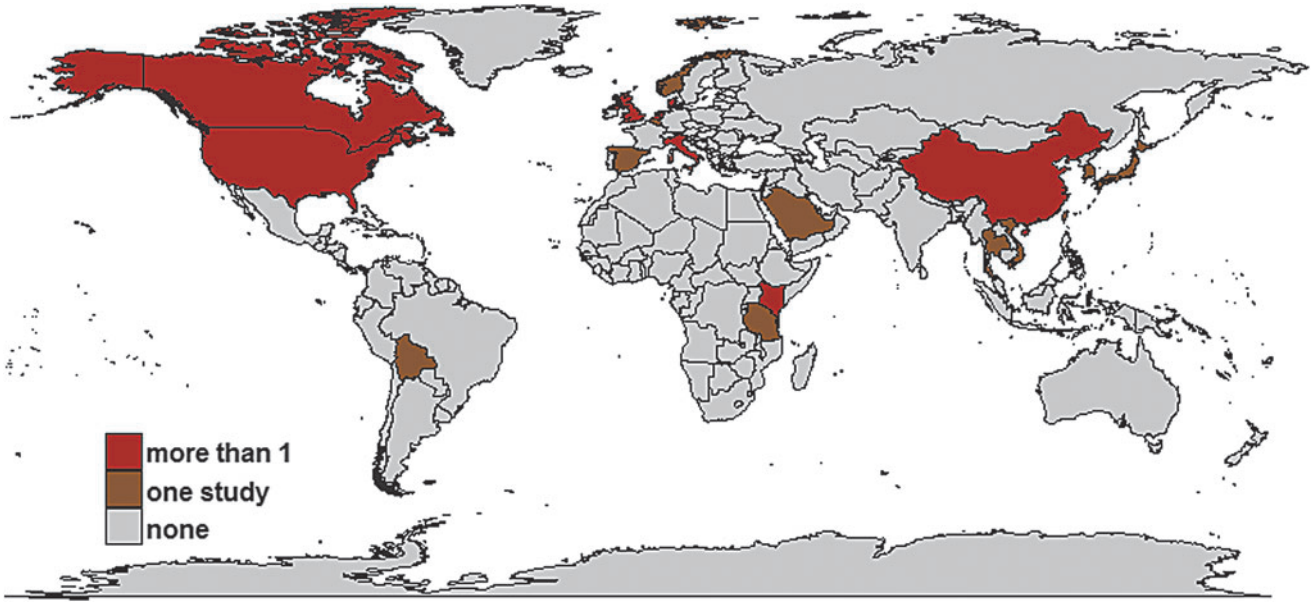

FIG. 2. Geographic distribution of included studies. Different colors show the number of articles from each country. The map was created using several R packages [ggplot2 (Wickham *et al.*, 2013), mapdata (Becker and Wilks, 2016), maps (Becker and Wilks, 2017), and ggmap (Kahle and Wickham, 2013)] in R version 3.4.1. The shapefile with borders of countries is freely available from the Natural Earth data set (www.naturalearthdata.com). Color images available online at www.liebertpub.com/fpd

Moodley and Guardabassi, 2009; Mulvey *et al.*, 2009; Smet *et al.*, 2009; Zhang *et al.*, 2009; Jakobsen *et al.*, 2010, 2011; Zhao *et al.*, 2010; Deng *et al.*, 2011; Vieira *et al.*, 2011; Stokes *et al.*, 2012; Ciccozzi *et al.*, 2013; Hu *et al.*, 2013; de Been *et al.*, 2014; Hammerum *et al.*, 2014; Valentin *et al.*, 2014; Dahms *et al.*, 2015; Dohmen *et al.*, 2015; Huijbers *et al.*, 2015; Lupindu *et al.*, 2015; Tseng *et al.*, 2015), and 12 studies (26%) did not suggest to find evidence supporting transmission between food animals and humans (Kariuki *et al.*, 1997, 1999; Maynard *et al.*, 2004; Kang *et al.*, 2005; Phongpaichit *et al.*, 2007; Graziani *et al.*, 2009; Schwaiger *et al.*, 2010; Xia *et al.*, 2010; Johnson *et al.*, 2012; Riccobono *et al.*, 2012; Jakobsen *et al.*, 2015; Ueda *et al.*, 2015). No study in our review suggested to provide evidence for AMR transmission from humans to food animals (Fig. 3).

Only one study (2%) based its conclusion regarding transmission on high resolution typing tools, 36 studies (80%) on intermediate resolution typing tools, six (13%) on low resolution typing tools, and two (5%) on co-occurrence of resistances (Fig. 3). Overall, 18 (40%) studies based their conclusion on transmission of AMR determinants, nine (20%) on transmission of AMR bacteria, and 18 (40%) transmission of AMR bacteria together with AMR determinants (Supplementary Fig. S4 in the Supplementary Data). We found no statistical association between whether direction of transmission was inferred and the nature of transmission ($p = 0.33$).

Studies suggesting to provide evidence of transmission of AMR from food animals to humans with direction specified

Three studies suggested to find evidence for transfer of AMR bacteria from food animals to humans, two of which concluded there is transfer of resistant clones from poultry to humans (Al-Ghamdi *et al.*, 1999; van den Bogaard *et al.*, 2001). In addition to overlapping clonal patterns, one study reported that human and chicken isolates were resistant to spectinomycin, an antibiotic mostly used in veterinary medicine (Al-Ghamdi *et al.*, 1999). Similarly, one study (van den Bogaard *et al.*, 2001) reported a higher prevalence of ciprofloxacin resistance among food animal isolates compared to human isolates.

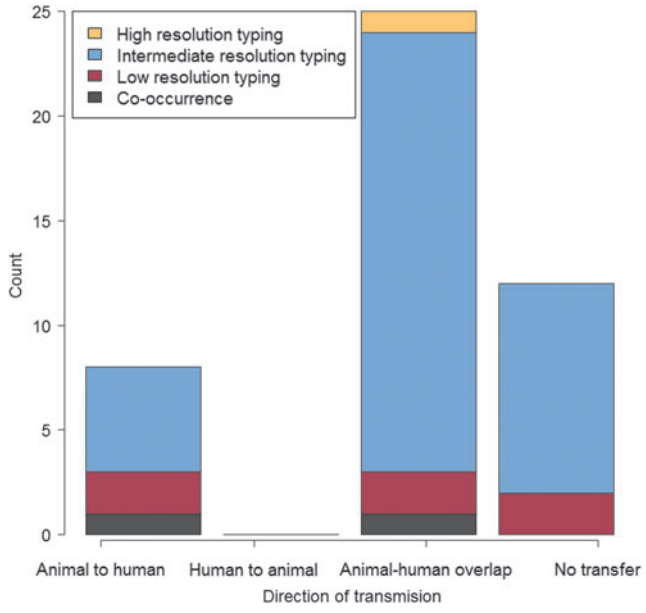

FIG. 3. Nature of evidence used to infer direction of transmission in each study. Color images available online at www.liebertpub.com/fpd

One study found identical ciprofloxacin-resistant isolates in chicken and humans, which they concluded was suggestive of food animal to human AMR transmission (Johnson *et al.*, 2006). Two studies suggested to find evidence for horizontal transfer of AMR determinants from food animals to humans (Hammerum *et al.*, 2006; Dierikx *et al.*, 2013). One study found that clonally unrelated poultry and human isolates shared ESBL/AmpC genes located on identical plasmid families (Dierikx *et al.*, 2013). Another study found that sulfonamide-resistant isolates from pigs and healthy humans shared sul1 and sul2 genes (Hammerum *et al.*, 2006).

Three studies suggested to support transmission of both AMR bacteria and their AMR determinants from food animals to humans. Two studies found similar sequence types, plasmid families and ESBL genes in *E. coli* isolates sourced from poultry and human patients (Leverstein-van Hall *et al.*, 2011; Giufre *et al.*, 2012). A further study reported an increase in tetracycline-resistant *E. coli* in humans in contact with tetracycline fed chicken and, therefore, suggested that chicken were a reservoir of AMR bacteria and plasmids for humans (Levy, 1978).

We found that studies suggesting to provide evidence of transmission of AMR from food animals to humans did not have distinct features compared to those suggesting overlap of resistance, with regard to study methodologies, food animal species, typing tools, or antibiotics tested. For most of these it is unclear why they suggested evidence of directional transmission when 25 broadly similar studies suggested only overlap of resistance.

Studies suggesting overlap indicating the possibility of between-host AMR transmission, with no direction specified

Four studies suggested there was evidence of overlap of resistant *E. coli* between humans and food animals. One of these studies found human and avian sequence types associated with multidrug resistance clustered together in a Bayesian phylogenetic tree (Ciccozzi *et al.*, 2013). Another study found indistinguishable PFGE patterns of ampicillin and tetracycline-resistant isolates in cattle and humans (Lupindu *et al.*, 2015). A cluster analysis of *E. coli* phylogroups found that human, pig, and chicken isolates clustered together (Jakobsen *et al.*, 2010). One extensive ecological study reported a significant correlation between the prevalence of resistance in human and livestock isolates, for both cephalosporins and fluoroquinolones (Vieira *et al.*, 2011).

Thirteen studies suggested there was evidence of overlap of AMR determinants in human and food animal isolates. Of the 13 studies, one study based on WGS and plasmid reconstruction found that clonally unrelated human and poultry isolates carried ESBL genes encoded on genetically identical plasmids (de Been *et al.*, 2014). Eleven studies found that unrelated human and food animal isolates shared identical AMR genes, integrons and plasmids (Oppegaard *et al.*, 2001; Winokur *et al.*, 2001; Ho *et al.*, 2009, 2010; Moodley and

Guardabassi, 2009; Mulvey *et al.*, 2009; Smet *et al.*, 2009; Zhang *et al.*, 2009; Stokes *et al.*, 2012; Huijbers *et al.*, 2015; Tseng *et al.*, 2015). One study identified identical plasmids encoding chloramphenicol resistance in unrelated human and food animal isolates (Jorgensen, 1983).

Eight studies suggested there was evidence of overlap of resistant *E. coli* and AMR determinants, with five of these finding that clonally related human and food animal isolates harbored similar ESBL gene types and plasmid types (Hu *et al.*, 2013; Hammerum *et al.*, 2014; Valentin *et al.*, 2014; Dahms *et al.*, 2015; Dohmen *et al.*, 2015). Likewise, two studies found that clonally related human and food animal isolates carried similar fluoroquinolone AMR genes (Zhao *et al.*, 2010; Deng *et al.*, 2011). In one study, cluster analysis of AMR gene profiles and *E. coli* pathotypes showed that human and food animal isolates clustered together (Jakobsen *et al.*, 2011).

Studies suggesting no evidence of transmission of AMR between humans and food animals

Two studies found no evidence for transfer of resistant clones, with one of these studies finding that human and avian ciprofloxacin-resistant *E. coli* strains had distinct phylogenetic compositions (Graziani *et al.*, 2009). Likewise, a PFGE analysis of multidrug-resistant *E. coli* isolates from sympatric children and chicken found that the isolates were source specific (Kariuki *et al.*, 1999).

Three studies reported no evidence for transfer of AMR determinants between food animals and humans with one of these studies reporting that human and porcine isolates had different distribution patterns of sulfonamide and tetracycline resistance genes (Schwaiger *et al.*, 2010). Two studies (Kariuki *et al.*, 1997; Phongpaichit *et al.*, 2007) reported that human and food animal multidrug-resistant isolates had distinct plasmids and integrons.

Seven studies reported no evidence for transmission of bacterial clones together with AMR determinants between food animals and humans. These studies showed that human and food animal isolates belonged to different phylogenetic groups, and had different AMR genes and plasmid profiles (Maynard *et al.*, 2004; Kang *et al.*, 2005; Xia *et al.*, 2010; Johnson *et al.*, 2012; Riccobono *et al.*, 2012; Jakobsen *et al.*, 2015; Ueda *et al.*, 2015).

Discussion

We performed a systematic review to explore the evidence that food animals are responsible for the transfer of AMR *E. coli* and their AMR determinants to humans. Some studies in our review suggested to provide evidence for the transfer of AMR from and between food animals and humans, while a larger number did not suggest to provide evidence of transmission in either direction. In addition to the differing nature of methods used to infer direction, studies in our review differed in sampling methodologies and antibiotics tested. These differences may have affected the conclusions made regarding the epidemiological connection between food animals and humans.

Much of the evidence regarding transfer of AMR was based on the demonstration that AMR *E. coli* clones and AMR determinants were indistinguishable in both food animal and human isolates. However, the demonstration of overlapping patterns should be interpreted with care as the direction of transmission is difficult to infer, and co-colonization from a shared source is also possible. Demonstrating the direction of transmission and thus the epidemiological history of pathogens and their determinants requires a quantitative description of relatedness, including phylogenetic analysis (Grad and Lipsitch, 2014).

Molecular techniques, such as MLST and PCR, used in most studies in our review, are limited in resolution (Didelot *et al.*, 2014). In one study, *E. coli* isolates were considered genetically indistinguishable based on MLST suggesting clonal transfer (Leverstein-van Hall *et al.*, 2011); however, subsequent WGS revealed that the isolates were genetically distinct (de Been *et al.*, 2014), highlighting the need for sequencing the entire genome, rather than only a few loci. WGS provides the current ''gold standard'' resolution for studying genetic relatedness, but as it is a technology that has only recently become routinely available it was used in just one study in our review. Future studies in this area could benefit from combining phylogeographic methods with WGS, which yields the potential for quantitative hypothesis testing for inferring pathogen movement between host populations (De Maio *et al.*, 2015; Woolhouse *et al.*, 2015).

Just over half of the studies in our review did not consider spatiotemporal relationships between human and food animal isolates, a fundamental requirement for investigating transmission (Singer *et al.*, 2006). Future research on the directionality of transmission will benefit from designing studies in which epidemiologically linked human and food animal populations are systematically sampled, preferably longitudinally (Woolhouse *et al.*, 2015). Moreover, there is considerable diversity within both human populations (i.e., healthy individuals vs. hospitalized patients) and food animals (i.e., free range vs. intensive farming) and the specific population considered may impact their exposure to diverse groups of bacteria; thus we recommend that future studies investigating transmission of AMR between humans and food animals clearly clarify the subpopulations studied. In addition, inclusion of detailed data on antibiotic usage in these populations should be considered.

None of the included studies provided a detailed overview of antibiotic usage in either human or food animal populations, or association between antibiotic usage and subsequent development of AMR. A recent systematic review has indicated that interventions that limit antibiotic use in food animals are associated with a reduction of AMR development in humans (Tang *et al.*, 2017), and therefore further research is warranted to explore this complex association.

Although transfer of AMR from humans to food animals is likely (Barber, 2001; Wooldridge, 2012), none of the studies in our review suggested to find evidence to support transmission from humans to animals. In many instances, responsibility for the burden of AMR has been placed on food animals (Barber, 2001; Woolhouse *et al.*, 2015; Mendelson *et al.*, 2017), and thus study bias may exist in terms of source attribution. Therefore, more research is needed to provide evidence for this potential route of transfer and, importantly, the relative magnitude of that spread.

Akin to the studies in our review, most AMR studies focus on a single bacterial type; however, rapid dissemination of AMR determinants frequently occurs between bacterial species, making it hard to track infection source (Sheppard *et al.*, 2016). Tracking these determinants, frequently located

on plasmids, using traditional molecular techniques may be limited. Using long read sequencing technologies such as Pacbio can overcome this by accurately generating plasmid structures (Orlek *et al.*, 2017).

Our systematic review excluded studies focusing on transmission of resistant bacteria and/or their AMR determinants through food animal-sourced food products. However, we acknowledge the potentially significant role played by food products of food animal origin in dissemination of AMR as reported in a recent systematic review (Lazarus *et al.*, 2015).

We have highlighted studies that suggest to provide evidence for transfer of resistant *E. coli* and their AMR determinants from food animals to humans. However, differences in study methodologies, such as lack of spatiotemporal overlap in sample collection, and the quality of typing tools used, suggest that transmission may occur, the evidence used to support the hypothesis is rarely compelling. The underlying problem is that demonstrating similarity or identity of AMR bacteria and/or AMR resistance determinants does not, by itself, provide information on directionality of transfer; this could be in either direction, or both, or neither but from a different source. Information on differential prevalence of resistance, and consumption of antibiotics, in the two populations may make stronger inference possible, but these data are rarely available.

Taken together, by combining genomic data analysis and epidemiological approaches it may be possible to reconstruct the complex transmission dynamics of resistant bacteria and their AMR determinants between human and food animal populations. Although we still have some way to go before a truly comprehensive integration of data—differential antibiotic usage data, detailed denominator data, information about the origin of the samples, human-food animal contact data, and pathogen sequence data—is available, disentangling and quantifying transmission of resistant bacteria and their AMR determinants between humans and food animals may still be an attainable goal.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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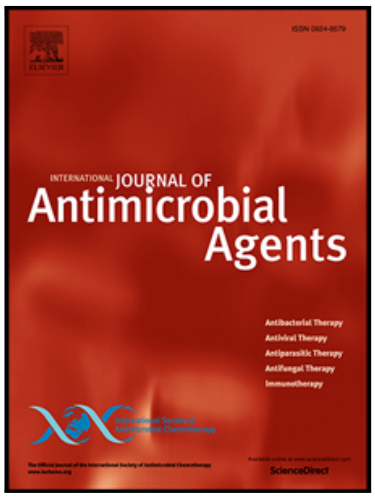

Epidemiology of antimicrobial resistant Escherichia coli carriage in sympatric humans and livestock in a rapidly urbanising city

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Highlights

- First ever study of AMR phenotypes in humans and urban livestock
- Highest AMR carriage in humans, pigs and poultry
- AMR more common in larger households
- Urban livestock keeping is not a risk factor for AMR in humans
- Use of animal manure affects the risk factor of AMR in humans

Epidemiology of antimicrobial resistant *Escherichia coli* carriage in sympatric humans and livestock in a rapidly urbanising city

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Abstract

There are substantial limitations in our understanding of the distribution of antibiotic resistance (AMR) in humans and livestock in developing countries. Here, we present the results of an epidemiological study examining patterns of AMR in *Escherichia coli* isolates circulating in sympatric human (n=321) and livestock (n=633) samples from 99 households across Nairobi, Kenya. *E. coli* isolates were tested for susceptibility to 13 antimicrobial drugs representing 9 antibiotic classes.

We detected high rates of AMR, with 47.6% and 21.1% of isolates displaying resistance to ≥ 3 and ≥5 antibiotic classes respectively. Human isolates showed higher levels of resistance to sulfonamides, trimethoprim, aminoglycosides and penicillins compared to livestock ($p<0.01$), while poultry isolates were more resistant to tetracyclines ($p=0.01$) compared to humans. The most common co-resistant phenotype observed was to tetracyclines, streptomycin and trimethoprim (30.5%). At the household level, AMR carriage in humans was associated with human density ($p<0.01$) and the presence of livestock manure ($p=0.03$), but livestock keeping on its own had no influence on human AMR carriage ($p>0.05$).

Our findings revealed a high prevalence of AMR *E. coli* circulating in healthy humans and livestock in Nairobi, with no evidence to suggest that keeping livestock, when treated as a single risk factor significantly contributed to the burden of AMR in humans, although the presence of livestock waste was significant. These results provide an understanding of the broader epidemiology of AMR in complex, and interconnected urban environments.

Keywords: Antibiotic resistance; AMR; *Escherichia coli;* One Health; Surveillance

1. Introduction

Antimicrobial resistance (AMR) in bacteria is regarded as one of the most serious public health threats of this century [1-3]. Over the last decade, increasing levels of resistance to clinically relevant antibiotics – including carbapenems [4] and colistin [5], which are considered antibiotics of last resort – has been reported in both human and animal populations.

Although *E. coli* can be a harmless gut commensal, some pathogenic strains can cause life-threatening bloodstream infections, and other common illnesses, such as urinary tract infections [6]. *E. coli* can also cause disease in animals, leading to severe economic losses due to mortality and morbidity [7]. Recently, *E. coli* was categorised by the World Health Organization as a priority pathogen due to its widespread antibiotic resistance [8].

Livestock have been implicated as a reservoir for AMR bacteria that may spread to humans, with the keeping of livestock widely believed to be a risk factor for AMR in humans [9, 10]. However, quantitative evidence describing the role of livestock in the emergence and transmission of AMR bacteria to human populations is lacking [11], particularly in low- and middle-income countries (LMICs) [12]. In the absence of routine surveillance of AMR in most LMICs, understanding the epidemiology of AMR is key to developing effective strategies targeting a reduction in the emergence and spread of resistance in the future.

To date, studies investigating the epidemiology of AMR have tended to focus on either human or livestock populations without making comparisons of resistances between the two populations. A recent systematic review [11] of studies investigating the link of AMR *E. coli* between humans and livestock found only 22 studies of spatiotemporally-related isolates from human and livestock populations, just six of which were conducted in LMICs. Notably, none of these studies considered urban livestock, which are increasingly important, particularly in LMIC settings [13] and may contribute to the maintenance of zoonotic bacteria and AMR in the complex urban environment [14].

This study focuses on the role of livestock keeping as a potentially high-risk urban interface for AMR transmission between humans and livestock in urban Nairobi. Nairobi is a rapidly growing city where livestock are commonly kept within household compounds, bringing them into close

contact with people. *E. coli* is an ideal organism to study the spread of AMR in this complex environment since it is a ubiquitous commensal in both livestock and humans, but with a wide range of resistance phenotypes.

Here, we report the first study characterising the patterns and epidemiology of antibiotic resistant *E. coli* from co-habiting human and livestock populations in a low resource urban setting. At the scale of individual households, we explore the role of livestock as risk factors for AMR carriage in humans, hence providing insight into the pathways of AMR transfer.

2. Methods

2.1. Study design

A cross-sectional study targeting sympatric human and livestock populations in Nairobi, Kenya was carried out from August 2015 to October 2016 as part of the Urban Zoo Project [15]. Briefly, Nairobi city was stratified into administrative sublocations according to socioeconomic status, identifying 70 possible sub-locations. Thirty three sub-locations were chosen with the aim of maximising spatial distribution, socio-economic diversity, and attempting to capture the diversity of livestock keeping practices across the city [15]. For each sub-location, three households – two livestock keeping (small livestock only (poultry, rabbits and goats), and large livestock (cattle and pigs) with or without small livestock) and one non-livestock-keeping – were selected at random within the dominant housing type.

A total of 99 households were involved in the study (Figure 1). Study design is explained in detail in the electronic supplementary material.

2.2. Sample collection and antimicrobial susceptibility testing (AST)

In each household, a questionnaire was used to collect data on household composition, socio-economic variables, livestock ownership, food consumption and medical history. Human and animal faecal samples were collected and transported on ice to one of two laboratories (University of Nairobi or Kenya Medical Research Institute) within 5 hours of collection. Samples were enriched in buffered peptone water for 24 hours, and thereafter plated onto eosin methylene blue agar (EMBA) and incubated for 24 hours at 37°C. One colony from each plate

was selected and sub-cultured for a further 24 hours on a second round of EMBA. Subsequently, one purified colony from each plate was selected at random (hereafter referred to as an 'isolate'), and confirmed as *E. coli* by biochemical testing, using triple sugar iron agar, Simmon's citrate agar, and motility-indole-lysine media.

Antimicrobial susceptibility testing for 13 antibiotics – ampicillin (10 μg/ml), amoxicillin-clavulanic acid (30 μg/ml), cefepime (30 μg/ml), cefotaxime (30 μg/ml), ceftazidime (30μg/ml), chloramphenicol (30 μg/ml), ciprofloxacin (5 μg/ml), gentamicin (10 μg/ml), nalidixic acid (30 μg/ml), streptomycin (25 μg/ml), sulfamethoxazole (30 μg/ml), tetracycline (30 μg/ml), and trimethoprim (2.5 μg/ml) – that are frequently used in either/both veterinary and/or human medicine in Kenya was carried out using the Kirby-Bauer disc diffusion method (Oxoid Ltd., Basingstoke, United Kingdom). Standardised protocols were used, in which antibiotic discs were dispensed onto bacteria-containing agar plates and incubated for a maximum of 18 hours at 35^{o}C. *E. coli* ATCC 25922 was used as a quality control of the susceptibility tests.

Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for *Enterobacteriaceae* [16] were used to determine breakpoints for classifying isolates as either susceptible ('susceptible' or 'intermediate') or non-susceptible ('resistant') for eleven of the 13 drugs. For tetracycline and trimethoprim, we classified isolates into resistant or susceptible because examination of the distributions of the zones of inhibition showed populations of isolates with distinct phenotypic resistance patterns (see electronic supplementary material, table S1). To describe multidrug patterns, the overall resistance profile was calculated by combining the resistance phenotype to each individual class, and thus antibiogram length (hereafter also referred to as AMR carriage) is the total number of antibiotic classes to which an isolate was phenotypically resistant.

2.3. Statistical analysis

The distribution of resistance phenotypes between hosts was calculated using Chi-squared tests (humans and livestock), and an one way ANOVA (human vs different livestock groups). Tukey's multiple-comparison test was performed *post-hoc* for pairwise comparisons between groups, and P values of <0.05 were considered significant.

Generalized linear mixed models (GLMMs), implemented in R package 'lme4' [17], with antibiogram length as the dependent variable were used to test whether AMR carriage differed between host groups. To investigate the co-occurrence of AMR phenotypes, a pairwise co-occurrence matrix (presence and absence) of the phenotypes was constructed using polycor package [18] in R and the co-occurrence relationships visualized using corrplot [19]. A correlation between two AMR phenotypes was considered statistically significant if the P-value (adjusted for multiple testing using Bonferroni correction) was <0.05.

To investigate finer scale household-level risk factors for AMR carriage in humans, we fitted a Poisson-distributed GLMM, with the counts of resistance phenotypes (antibiogram length) as the response variable. Model explanatory variables were human density (count of people in a household as a function of household area) and types of livestock kept in the household (small livestock only, large livestock with or without small livestock, and no livestock). Additionally, for households that kept livestock, a separate Poisson-distributed GLMM was fitted to investigate the effect of human density and manure disposal practises (manure disposed in the household compound or outside) on human antibiogram length. Separate models were fitted for the most prevalent AMR phenotypes (tetracyclines, aminoglycosides, sulfonamides, penicillins, and trimethoprim).

To account for the nested (or hierarchical) nature of our sampling design household site (n=99), sublocation (n=33) and wealth category (n=7) were included as random factors. Further details of data exploration and statistical models are given in the electronic supplementary material.

3. Results

A total of 954 isolates composed of 321 human and 633 livestock *E. coli* isolates in Nairobi, Kenya, were analysed. The number of isolates obtained from each source is presented in Table 1.

3.1. Patterns of antimicrobial resistance in humans and livestock

The most common resistance phenotypes (>40% of resistant isolates) were to sulfonamides, trimethoprim, tetracyclines, and aminoglycosides. A smaller percentage of isolates (<10%) were

resistant to amoxicillin/clavulanic acid, cephalosporins, phenicols, and fluoroquinolones (Table 2 and Figure 2). The distribution of resistance to the individual drugs tested is given in Table s2.

When analysed by host, human isolates were more commonly resistant to each of the individual antibiotic classes, than those of animal origin. Of 321 human isolates, >40% were resistant to sulfonamides, trimethoprim, aminoglycosides, and tetracyclines. Of 633 livestock isolates, >40% of isolates were resistant to sulfonamides, tetracyclines and trimethoprim. For both human and livestock isolates, <10% of isolates were resistant to phenicols, fluoroquinolones, cephalosporins and beta-lactams. Resistance to penicillins, aminoglycosides, sulfonamides and trimethoprim was significantly more common in humans than in livestock ($p<0.01$, Chi-squared test; Table 2, Figure 2a).

The prevalence of resistance to penicillins, tetracycline, aminoglycoside, sulfonamides and trimethoprim varied significantly between humans and livestock stratified by taxonomic groups (poultry, pigs, rabbits, bovines and goats; Tukey's post hoc test). Humans were more likely to carry *E. coli* resistant to penicillins, aminoglycoside, sulfonamides and trimethoprim than all species of livestock ($p<0.05$, one-way ANOVA with Tukey's multiple-comparison test). Conversely, poultry were more likely to carry isolates resistant to tetracyclines than humans (Figure 2b, Supplementary Figure s1).

Overall, 284 (29.7%) isolates were susceptible to all 13 antibiotics tested (nine antibiotic classes). The proportion of pan-susceptible isolates was significantly higher among livestock isolates (n=217/633, 34.3%) than in human isolates (n=67/321, 20.9%) ($X^2=17.6$, $p<0.01$, Chi-squared test). Of the 217 pan-susceptible livestock isolates, 22% of poultry isolates (n=76), 51.6% of bovine isolates (n=33), 33.3% of pig isolates (n=17), 54.6% of goat isolates (n=72), and 46.3% of rabbit isolates (n=19) were pan-susceptible. Across both human and livestock isolates, 404 (47.6%) and 201 (21.1%) isolates were resistant to ≥ three and five antibiotic classes respectively. Eight isolates (0.8%) showed resistance to ≥7 antibiotic classes tested; five (1.6%) from humans and three (0.9%) from poultry (Figure 3).

Antibiogram length (i.e. the total number of antibiotic classes an isolate is resistant to) was significantly higher in humans than in livestock (OR=1.14, 95% CI 0·68 to 0·81, $p<0.01$, marginal

R^2 0·041, GLMM). However when studied in more detail, antibiogram lengths in human isolates were similar to those from pigs and poultry (p>0.05, marginal R^2 0.151, GLMM) but significantly higher than those from bovines, goats and rabbits (p<0.05, marginal R^2 0.151, GLMM) (Table 3, Figure 3).

Examination of the similarity of *E. coli* antibiograms from human and livestock isolates revealed 84 distinct profiles: 30 in livestock, 19 in humans and 35 common to both (Table s4). Using a co-occurrence analysis based on a statistically significant (p<0.05) correlation coefficient (ρ>0.5) we identified a tetracycline-sulfonamide-trimethoprim cluster (Figure 4). This co-resistance was identified in 340 isolates (30.5%): 115 (35.8%) humans and 225 (35.5%) livestock – 156 (45.2%) poultry, 24 (47.1%) pigs, 9 (22.0%) rabbits, 14 (21.9%) bovines, and 22 (16.7%) goats. There were no significant differences in the distribution of this profile between human and the other host groups (X^2<0.01, p > 0.98, Chi-squared test). Further, denoting multi-resistance, this cluster was commonly associated with resistance to aminoglycoside and penicillins.

3.2 Antimicrobial resistance exchange between humans and livestock at the household level

In any given household, we found no evidence that the presence of livestock increased risk of human AMR carriage (large livestock OR=0.94 p=0.24, 95%CI 0.72 to 1.22; small livestock OR=1.04, p=0.94, 95% 0.82 to 1.30, marginal R^2 0.3, GLMM) (Table 4). However, human antibiogram length increased with human density (OR=1.26, p=0.003, 95% CI 1.08 to 1.47, marginal R^2 0.3, GLMM) (Figure 5). The impact of livestock keeping on human AMR carriage was potentially influenced by disposal practices of animal manure: keeping manure inside the household perimeter, compared to disposing of it externally, was associated with greater human antibiogram length (OR=1.29, p=0.03, 95%CI 1.02 to 1.63, marginal R^2 0.5, GLMM) (Table 4). Our results were consistent when we performed separate analyses for the individual resistances (Table s3).

4. Discussion

In this study we applied ecological and epidemiological approaches to characterise the epidemiology of AMR *E. coli* isolates systematically collected from sympatric human and livestock populations in the rapidly developing urban landscape of Nairobi, Kenya.

Resistance to aminoglycosides, sulfonamides, tetracyclines, trimethoprim and penicillins was high in both humans and livestock, while resistance to cephalosporins and fluoroquinolones was low. These results are consistent with previous studies [20-23] and may in part be a reflection of the patterns of antibiotic use in human and animal health. However, background data on antimicrobial use in these populations is limited. Our results indicating a high prevalence of AMR carriage are based on non-clinical isolates from humans and livestock.

When analysed by host, human isolates appeared to have a higher prevalence of AMR carriage when compared to livestock isolates, with the exception of tetracyclines. In particular, the observed prevalence was significantly higher in four clinically relevant antibiotic classes (penicillins, sulfonamides and trimethoprim and aminoglycosides). A possible explanation for this variation in AMR carriage is that it relates to variation in antibiotic use between these populations. Although antibiotics are used extensively in both human and livestock populations, previous studies have shown that frequency of use of antibiotics is higher in human medicine than in livestock, especially in resource-poor settings [24, 25]. Similarly, in community settings where over-the-counter access to drugs is common, it is likely that humans have access to a broader range of antibiotics, either through self-medication or inappropriate prescribing; common practices in many low and middle-income countries (LMICs) [26, 27]. Likewise, in such settings, infections are commonly treated empirically (often using antibiotics) with limited microbiological investigations to ascertain the causal organism(s).

Although chloramphenicol use in food animals has been banned in Kenya [28], we noted 3% resistance to this antibiotic in livestock. This may be explained by the use of florfenicol, a fluorinated derivative of chloramphenicol, which shows some cross-resistance with chloramphenicol [29]. Similarly, the observed levels of resistance against ciprofloxacin (a quinolone antimicrobial not licensed for veterinary use) among livestock isolates is probably explained by cross-resistance with other quinolones used in veterinary medicine, such as enrofloxacin and norfloxacin.

At the household level, there is evidence of an intricate interplay between humans and livestock in relation to the development and transmission of AMR. Our analysis revealed that

human AMR carriage increased with number of occupants in a household, and that keeping manure inside the household compound was also significantly associated with AMR carriage in humans. In urban Nairobi, people live in a continuum of urban spaces with varying human and animal population densities, with the majority (>60%) of people living in slums [30, 31], environments characterised by small household areas and high population densities. Population density is an important factor in the population prevalence of AMR in populations [32], and may in part be due to the significant correlation between overcrowding and high infectious diseases burden more broadly[33]; an important driver of antibiotic use in resource poor settings such as Nairobi. Similarly, high human populations within a household result in a greater epidemiological connectivity; thus facilitating exchange of AMR bacteria and their AMR determinants. The number of urban dwellers in the majority of LMIC cities, including Nairobi, is projected to grow significantly in the near future [34]. While this urban demographic change is unfolding, disease burden is expected to burgeon, precipitating high antibiotic use. For this reason, measures to curb infectious diseases burden by the public health policy makers, in part to reduce drug pressure on micro-organisms, are needed.

Our results suggest that at the household level, livestock ownership in and of itself does not add to the risk of acquisition or carriage of AMR bacteria in humans. However, given the multiple pathways of AMR exchange between humans and livestock [35], via the food chain or due to environmental pollution, it is possible that the direct effect of livestock keeping on levels of AMR in humans could be confounded by other factors not captured in this study. Our study does, however, suggest that, whilst AMR carriage (antibiogram length) was not directly associated with the presence of livestock in the household, the impact of keeping livestock on human AMR carriage was mediated by some practices associated with livestock keeping, namely the presence or absence of animal manure in the household. These results support other studies that have identified animal manure as a reservoir of AMR bacteria and AMR determinants [36, 37]. Importantly, amplification and persistence of AMR determinants such as AMR plasmids can take place in manure and be further disseminated to humans via cross-contamination pathways such as through exposed water and food [38], or via peri-domestic wildlife. Although there is still a lack of knowledge concerning the exact mechanism, particularly

the genetic basis of transmission [39], strategies that limit AMR gene flow to and from manure (to humans) should be adopted. Such measures include safe disposal of manure from households, and manure pre-treatment prior to application onto crop farms where possible.

It is important to note that, while our analysis was not designed to address transmission of AMR bacteria and their AMR determinants, it is also plausible that clonal expansion could have played a role in the observed AMR patterns. Our finding of 35 common AMR profiles in both human and livestock bacterial populations may, in part, reflect overlapping antibiotic usage patterns, acquisition of AMR from a shared source or clonal expansion. We hypothesise that our finding that 30.5% (340/954) of all isolates contain a tetracycline-sulfonamide-trimethoprim cluster phenotype and that the pairwise correlations between these three antibiotic classes were very high is suggestive of a conjugative MDR plasmid circulating within the *E. coli* population in both human and livestock populations. AMR genes conferring resistance to tetracycline, sulfonamide and trimethoprim antibiotic classes are commonly associated with mobile genetic elements [40], and these elements play a pivotal role in dissemination of multidrug resistance in *E. coli* isolates. Genetic data is required to validate the existence of mobile genetic elements, and determine whether AMR genes are being transferred across them.

Distinguishing molecular transmission of AMR, from selection for AMR due to antibiotic use, is challenging [11]. In particular, in an urban environment such as Nairobi, where human habitation, livestock keeping, and food supply chains are interconnected [41] the relative contributions of the aforementioned drivers are difficult to quantify. At a finer scale, any study investigating the transmission of AMR between humans and livestock in these low resource settings needs to consider indirect transmission, rather than just direct animal to human and/or human to animal transmission. Whilst direct host-to-host transmission of AMR bacteria and AMR determinants may occur, in these intricate ecosystems, the role played by the wider environment (e.g. wildlife, soil and, in particular, hospital and farm effluents) in relation to acquisition of AMR from a common source may be vital.

5. Conclusion

Taken together, using a rigorously-structured epidemiological study design, we report a high prevalence of AMR *E. coli* carriage in livestock and humans outside the clinical setting across a developing-country urban landscape, with no evidence that direct contact with livestock contributes to the burden of human AMR, but that indirect contact between livestock and humans does play a role. In LMIC urban ecosystems, the elevated prevalence of AMR in both human and livestock populations could be attributed to unregulated access to antibiotics, poor hygiene and sanitation, and waste management, which encourage the evolution and spread of AMR bacteria. These findings highlight a need for targeted surveillance strategies across various sectors, and for actors to address and design effective measures to curb AMR in these populations, both in Nairobi and in other similar urban landscapes. Further work is required to understand the ecology of genetic determinants of resistance, in particular the extent of the role plasmids play in the dissemination and evolution of resistance traits in these human and livestock populations.

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Declarations

Funding: Study was supported by the UK Medical Research Council, Biotechnology and Biological Science Research Council, the Economic and Social Research Council, the Natural Environment Research Council

Competing Interests: None

Ethical Approval: The collection of data adhered to the legal requirements of the International Livestock Research Institute (ILRI). Ethical approval for human sampling and data collection was obtained from the ILRI Institutional Research Ethics Committee (ILRI IREC) ILRI-IACUC2015/09. ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federal wide Assurance (FWA) for the Protection of Human Subjects in the United States of America. Livestock samples were obtained under the approval of the ILRI Institutional Animal Care and Use Committee (reference ILR-IACUC2015/18).

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Figure Legends

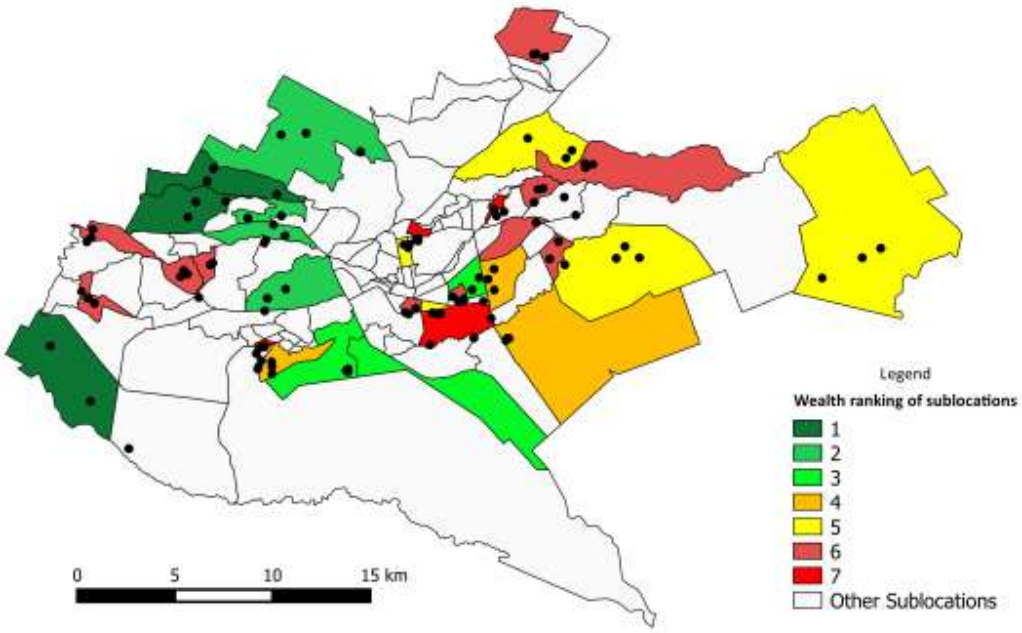

Figure 1. Map of Nairobi city, Kenya indicating the location of the sampled households (black dots) and 33 sublocations (coloured by wealth category; 1 – wealthy, 7 – poor).

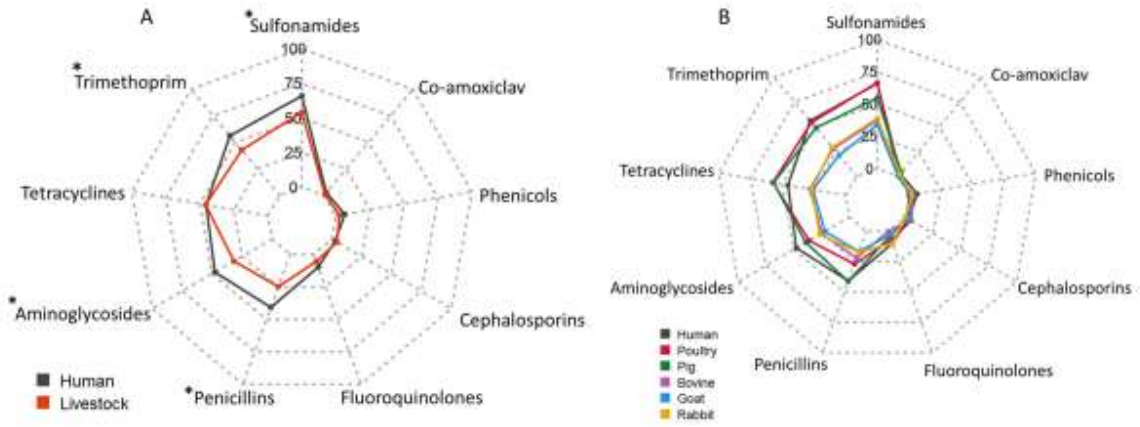

Figure 2. Radar charts showing percentages of *E. coli* isolates resistant to 9 antibiotic classes. a) human (n=321) and livestock (n=633), and b) human and the different livestock species' (poultry, pig, bovine, goat, rabbit). (Asterisks denote significant differences between carriage of this particular resistance phenotype in livestock and humans).

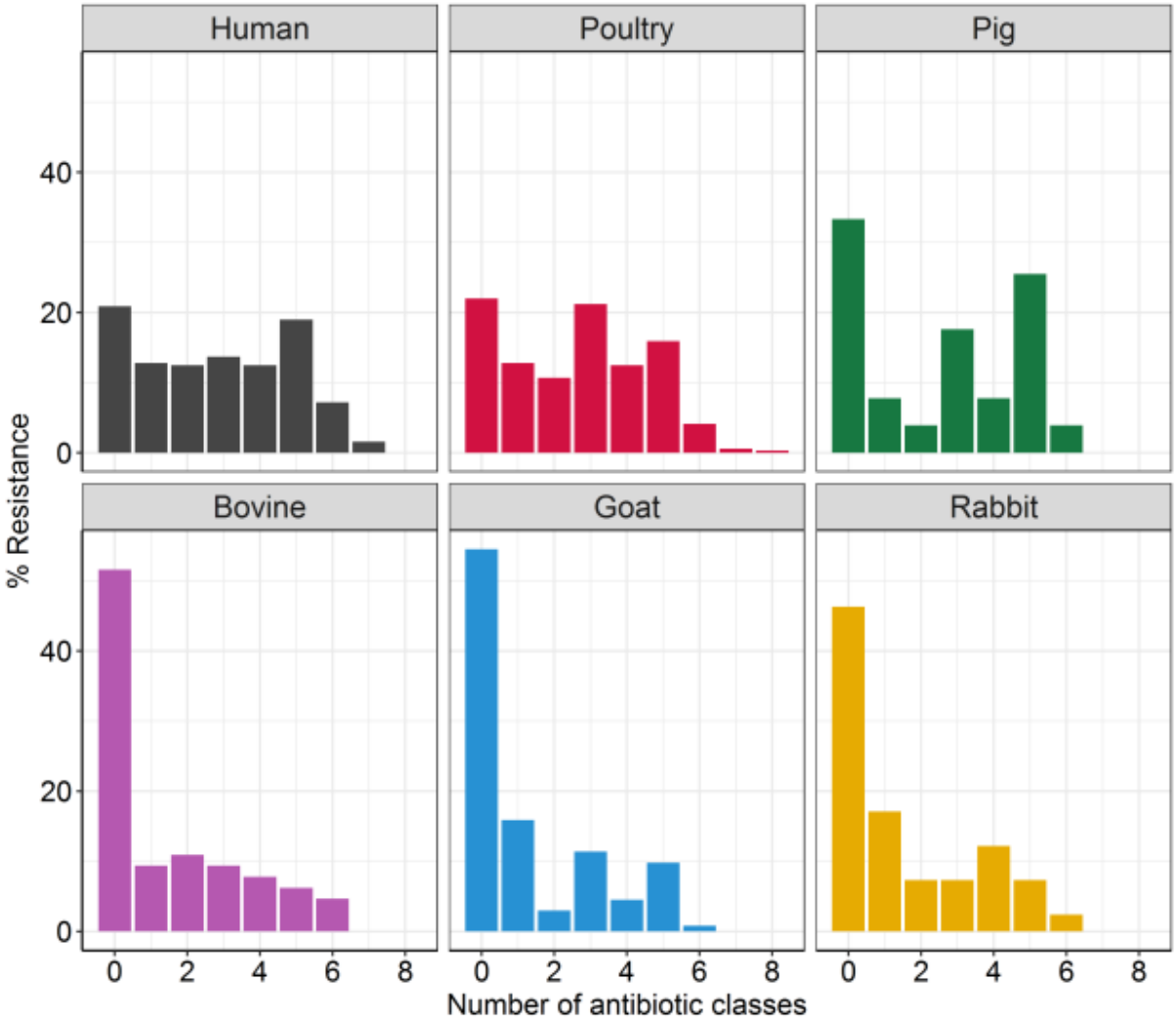

Figure 3. Distribution of multidrug resistance patterns among *E.coli* isolates obtained from humans (n=321), poultry (n=345), pigs (n=51), bovines (n=64), goats (n=132), and rabbits (n=41), in Nairobi, Kenya.

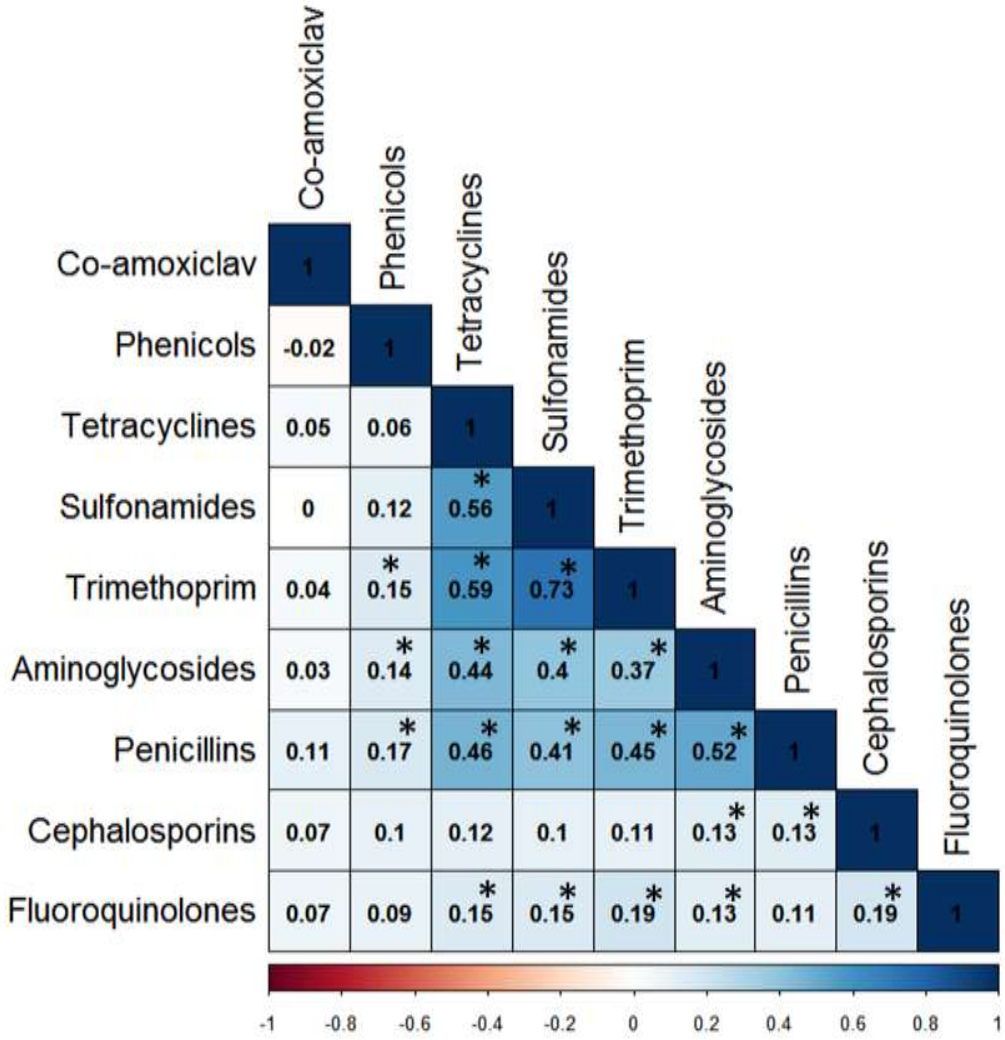

Figure 4. Heat map representing correlations among AMR phenotypes across human (n=321) and livestock (n=633) *E. coli* isolates. The boldness of the colour represents the strength of the relationship between phenotypes, with stronger correlations having bolder colours. Numbers within boxes represent correlation coefficient (r) values. * indicates statistically significant correlations ($p<0.05$). The scale bar at the bottom indicates whether the correlation between phenotypes is positive (closer to 1, dark blue) or negative (closer to −1, dark red).

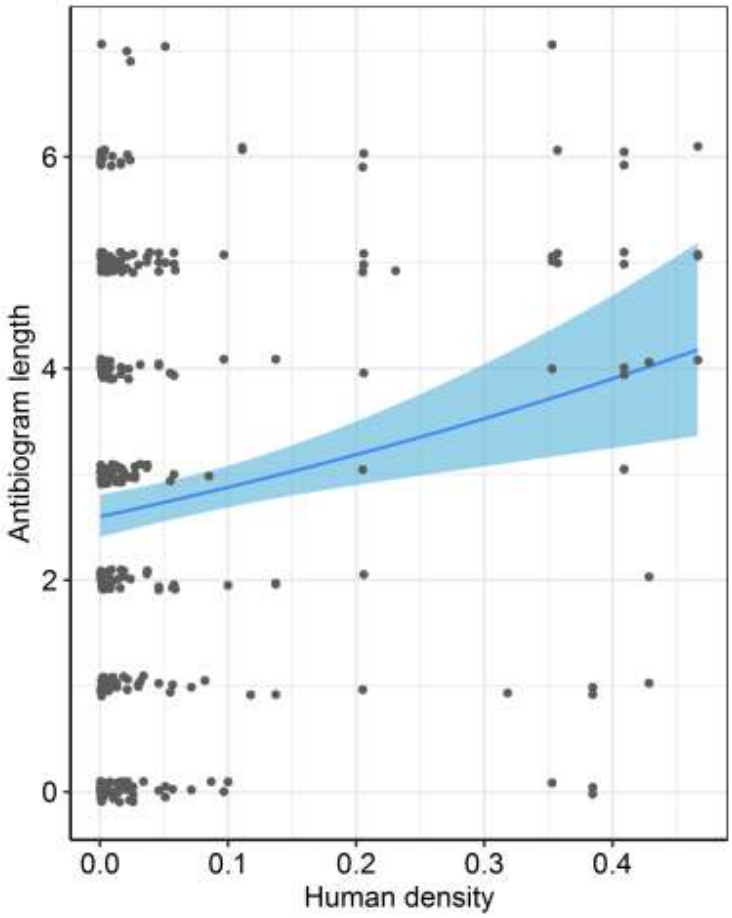

Figure 5. Fit of a Poisson generalised linear mixed effects model showing how increasing human density in a household influences the antibiogram length in humans. All other covariates in the models are kept constant. Shading on either side of each line represents 95% confidence intervals. Points have been jittered for clarity.

Table 1. Number of human and livestock isolates collected from the 99 households from Nairobi, Kenya (2015-2016). Livestock isolates are broken down by source.

Source	Number of isolates	% of isolates
Human	321	33.7
Livestock:		
Poultry	345	36.2
Bovine	64	6.7
Goat	132	13.8
Pig	51	5.3
Rabbit	41	4.3

Table 2. Percentages of *E. coli* isolates resistant to different antibiotic classes classified by host type (human or livestock). Numbers show percentages of isolates classified as resistant based on the zone of inhibition. Categorical interpretation is based on breakpoints used as described in the methods section. NS=Not Significant

Antibiotic category	Overall (n=954)	Human (n=321)	Livestock (n=633)	Adj. p value
Sulfonamides	58.2	66	54.2	0.005
Aminoglycosides	37.1	47.7	31.8	<0.001
Trimethoprim	47.3	56.1	42.8	0.001
Tetracyclines	45.7	45.5	45.8	NS
Penicillins	30.2	40.8	24.8	<0.001
β-lactam (co-amoxiclav)	1.5	2.5	0.95	NS
Phenicols	4.0	6.5	2.69	NS
Cephalosporins	3.8	2.8	4.27	NS
Fluoroquinolones	6.8	9.7	5.37	NS

Table 3. Results of a Poisson generalise linear mixed model examining the likelihood of AMR carriage within different host groups. Human is used as the reference level. NS =Not significant

	No of isolates	Estimate	Standard error	P value
Human	321	Reference	Reference	Reference
Livestock	633	-0.13	0.16	<0.01
Bovine	64	-0.28	0.14	0.03
Poultry	345	-0.08	0.05	NS
Pigs	51	0.08	0.11	NS
Rabbits	41	-0.37	0.16	0.02
Goats	132	-0.48	0.11	<0.01

Table 4. Results of two generalized Poisson Mixed Models investigating household risk factors for AMR carriage (antibiogram length) in humans at the household level. Households not keeping livestock used as the reference level in Model 1.

Model 1: Antibiogram length, humans in all households	**Estimate**	**Standard error**	**P value**
Human density	0.23	0.08	0.003
Large livestock (with or without small livestock)	-0.14	0.12	0.24
Small livestock only	0.0075	0.11	0.94
Model 2: Antibiogram length, humans in livestock keeping household only			
Human density	0.24	0.09	0.009
Manure in household	0.26	0.12	0.03

Electronic supplementary material:
The online version of this article contains supplementary material.

journal of global health

A cross-sectional survey of practices and knowledge among antibiotic retailers in Nairobi, Kenya

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Background Antimicrobial resistance (AMR) driven by antibiotic consumption is a growing global health threat. However, data on antimicrobial consumption patterns in low- and middle-income countries (LMICs) is sparse. Here, we investigate the patterns of antibiotic sales in humans and livestock in urban Nairobi, Kenya, and evaluate the level of awareness and common behaviours related to antibiotic use and AMR amongst human and veterinary pharmacists.

Methods A total of 40 human and 19 veterinary drug store pharmacists were interviewed in Nairobi in 2018 using a standard questionnaire. Data recorded included demographic variables, types of antibiotics sold, antibiotic customers, antibiotic prescribing practices and knowledge of antibiotic use and AMR.

Results Our study shows that at the retail level, there is a considerable overlap between antibiotic classes (10/15) sold for use in both human and veterinary medicine. Whilst in our study, clinical training significantly influenced knowledge on issues related to antibiotic use and AMR and respondents had a relatively adequate level of knowledge about AMR, several inappropriate prescribing practices were identified. For example, we found that most veterinary and human drug stores (100% and 52% respectively) sold antibiotics without a prescription and noted that customer preference was an important factor when prescribing antibiotics in half of the drug stores.

Conclusion Although more research is needed to understand the drivers of antibiotic consumption in both human and animal populations, these findings highlight the need for immediate strategies to improve prescribing practices across the pharmacists in Nairobi and by extension other low- and middle-income country settings.

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Antibiotic resistance (AMR) has been described as one of the most serious public health threats of this century [1-3]. Antimicrobial use, misuse and overuse in human and animal medicine exerts an important selective pressure for AMR. Global antimicrobial use in human and food animals is increasing, mainly due to increased disease burdens and expanded intensive livestock production respectively [4].

As in most cities in low and middle income countries (LMICs), in urban Nairobi the high incidence of bacterial diseases and antimicrobial resistance in clinical medicine is a major public health challenge [5]. In both human and animal populations, antibiotics are used for both prophylaxis

and treatment of infectious diseases and many of the antibiotics used to treat these infectious diseases are pharmacologically similar. It is estimated that more than half of all antibiotics (for use in both humans and animals) are purchased without a prescription and used over-the-counter [6]. There is a paucity of data in Kenya regarding antibiotic usage at both the national and the regional level, but there have been attempts to assess the consumption of antibiotics in food producing animals and human health using sales data [7]. These studies, based on antibiotic import data, estimate that, from 1997-2001, consumption of antibiotics in clinical medicine increased by 4%, with penicillins and fluoroquinolones being the most widely used antibiotics. Collecting data on antibiotic use simultaneously in both animals and humans could provide essential data to help disentangle the primary drivers for the development of antibiotic resistance. Here, we carried out a survey to investigate the patterns of antibiotic sales in humans and animals in urban Nairobi.

Pharmacists (both human and veterinary) play a pivotal role in enhancing antimicrobial stewardship initiatives, not just by highlighting the AMR problem, but also by influencing crucial prescribing decisions [8,9]. To further improve antibiotic use and antibiotic stewardship programmes it is important to have an understanding of the knowledge and attitudes towards antibiotics within different populations such as pharmacists. At present, there has been limited research in understanding pharmacists' knowledge of antibiotic resistance. Here, we aimed to assess the level of awareness and common behaviours related to antibiotic prescribing amongst human and veterinary pharmacists.

MATERIALS AND METHODS

Study design and setting

A cross-sectional study targeting human and veterinary drug stores in urban Nairobi, Kenya was carried out in January 2018 as part of the UrbanZoo project [10]. Briefly, Nairobi County was classified into seven wealth categories according to average income, identifying 70 possible sub-locations. Thirty-three sub-locations were chosen for sampling with the aim of maximising spatial distribution, socio-economic diversity, and attempting to capture the diversity of livestock keeping practices across the city [10]. Within each of the pre-selected 33 sub-locations we randomly selected and visited two community drug stores – one veterinary drug store and one human drug store. The final distribution of sampled human and veterinary drug stores is shown in **Figure 1**. Community human drug stores are mostly operated by pharmaceutical technicians who are responsible for dispensing antibiotics, while only a few, mostly large, drug stores have a registered pharmacist (holding a bachelor's degree in pharmacy). Both pharmaceutical technicians and pharmacists are able to sell, but not prescribe antibiotics in Kenya [11]. Veterinary drugs stores are mostly operated by animal health technicians (also referred to as para-veterinarians) with just a few operated by veterinarians. Animal health technicians are also not allowed to prescribe antibiotics. All of the above-mentioned groups will have obtained clinical/veterinary training at varying levels. In this study, we define 'pharmacist' as someone selling antibiotics in a veterinary or a human drug store irrespective of the level of clinical training.

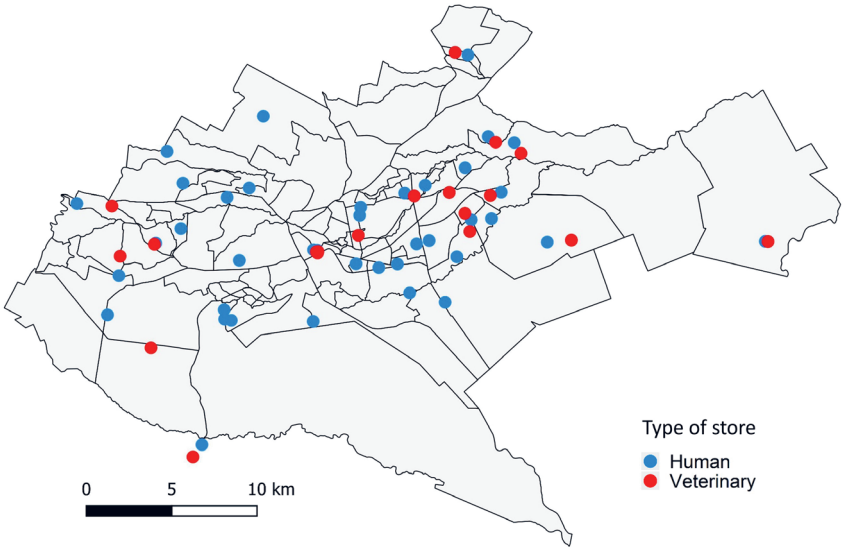

Figure 1. Map of Nairobi county indicating locations of study sites.

A draft of the questionnaire was pre-tested with five drug stores (three human and two veterinary), and refined on basis of the feedback from the pre-testing sessions before final dissemination. In each drug store a detailed questionnaire was used to collect data on socio-demographic variables, training on antibiotic use, types of antibiotics sold (by class), the four antibiotic classes most commonly sold, variation in antibiotic sales, antibiotic sources, antibiotic customer characteristics, and antibiotic

prescribing practices. A summary of the collected data are presented in Table S1 in **Online Supplementary Document**. To assess the knowledge of antibiotic use and AMR amongst the human and veterinary pharmacists we adapted a standard questionnaire prepared and used by the World Health Organization [12]. First, we assessed respondents' knowledge on a number of terms routinely used to describe the problem of antimicrobial resistance. These terms included: antibiotic resistance, superbugs, antimicrobial resistance, AMR, drug resistance and antibiotic-resistant bacteria. Next, respondents were asked about their level of agreement with ten statements describing their knowledge on AMR and potential solutions to antimicrobial resistance. The statements were written on a 5-point Likert scale [13].

Data analysis

Descriptive statistics were prepared for all data including frequencies and percentages for categorical variables (eg, gender and education level) and means, medians, standard deviations (SDs), quartiles, and ranges for quantitative variables (eg, number of customers) depending on the distribution of the data. We used a χ^2 or Fisher exact test using R package stats [14] to describe differences between proportions of clinical training (present or absent) by type of drug store (human or veterinary) and Mann-Whitney U test to compare range of antibiotics (number of different antibiotic classes) in the two types of drug stores.

Prescribing practices

Next, we aimed to describe practices and evaluate the factors associated with drug prescribing amongst human and veterinary pharmacists. To achieve this, we collected data on information provided to customers after purchasing antibiotics as an indicator of good prescribing practices. This included: whether pharmacists provided customers with information on dosage, directions for use (ie, completing the prescribed dose), storage instructions, side effects, expiry date and contra-indications [15]. The data were then assessed for multicollinearity using the corrplot package [16] in R to determine if answers to any two or more questions were correlated. There did not appear to be a sufficiently strong correlation between any two questions for any of them to be excluded. To derive a measure of prescribing practices amongst the respondents we developed a composite score (sum of the binary variables, 0/1) from individual indicators of good prescribing practices. We fitted a generalized linear model (GLM) using R package lme4 [17] to assess possible influence of type of drug store (human/veterinary), clinical/veterinary training (present or absent), education level (high or low) and range of antibiotics sold (number of different antibiotic classes) in the drug store (proxy for store size) on the composite prescribing practices score. We analysed clinical/vet training (defined as having a degree or diploma in clinical or veterinary medicine) and education level separately as some pharmacists had received training in disciplines not related to medicine or veterinary studies. We considered $P<0.05$ to be statistically significant.

Knowledge on antimicrobial resistance

In order to assess the internal consistency of the ten statements evaluating the level of knowledge on AMR, Cronbach's alpha coefficients were calculated for each statement. Internal consistency is a measure of item-total correlations and reliability of the scale, thus describing the extent to which all items in a test measure the same concept or construct [18]. An unstandardized Cronbach's alpha coefficient of 0.7 or above was considered to demonstrate adequate reliability.

Principal Component Analysis (PCA) using polychoric correlation [19] was used to generate a composite index for knowledge score and to investigate clustering of the knowledge statements [20]. Analyses were performed using the psych package [21] to conduct PCA (using the principal function) without rotation of axes. Scree plot inspection [22] and parallel analysis [23] were used to choose the optimal model in terms of number of components to retain.

The scores of the first PCA component were used as measure of knowledge of AMR, and the higher the knowledge score, the higher the implied knowledge of AMR of that respondent. A generalised linear model was used to investigate the possible influence of type of drug store (human or veterinary), clinical training (present or absent), education level (high or low), and range of antibiotics sold in the drug store (proxy for store size) on the level of knowledge of AMR.

Ethical approval

Ethical approval for this study was obtained from the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (ILRI IREC) (project reference: ILRI-IREC2017-35).

Table 1. Participant demographics and baseline clinical characteristics

Characteristic	Human drug stores	Veterinary drug stores
Number of individuals	40 (67%)	19 (33%)
Gender:		
Female	21 (52.5%)	9 (47.4%)
Male	19 (47.5%)	10 (52.6%)
Highest education level:		
Primary	0	1 (5.2%)
Secondary	4 (10%)	5 (26.3%)
Certificate	4 (10%)	5 (26.3%)
Diploma	24 (60%)	7 (36.8%)
Degree	8 (20%)	1 (5.3%)
Role:		
Owner	11 (27.5%)	5 (26.3%)
Worker	29 (72.5%)	14 (73.7)
Age (median)	30	30
Clinical/veterinary training:		
Present	36 (90%)	11 (57%)
None	4 (10%)	8 (42.1%)
Source of training on antibiotic stewardship and AMR:		
Clinical training only	16 (40%)	3 (15.8%)
CPD	20 (50%)	8 (41.1%)
None	4 (10%)	8 (42.1%)

AMR – antimicrobial resistance, CPD – continuing professional development

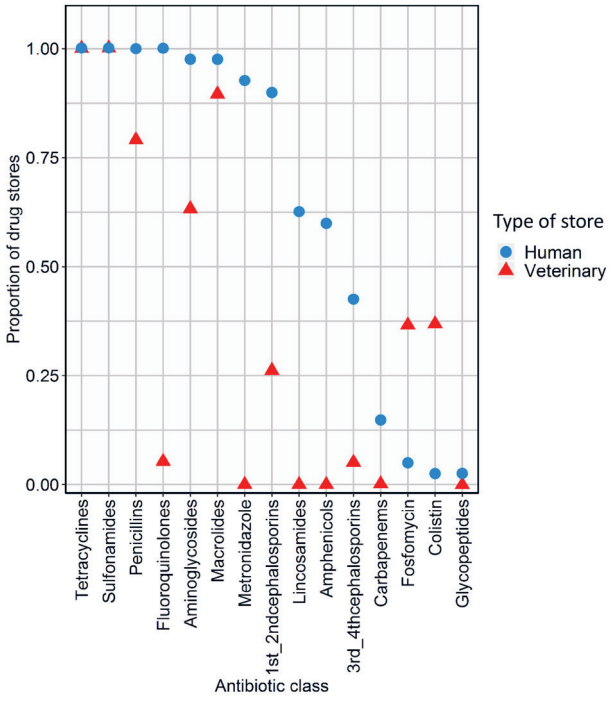

Figure 2. Proportion of the 15 antibiotic classes reported in human (n = 40) and/or veterinary drug stores (n = 19). Data arranged in order of the average proportion of antibiotic classes.

RESULTS

Demographic data about the respondents

A total of 59 participants were interviewed – 40 from human drug stores and 19 from veterinary drug stores (**Table 1**). Some sub-locations did not have a veterinary drug store as these tend to be located in zones of the city where animals are kept. The median age of participants in both human and veterinary stores was 30 years (range; human, 21-51; livestock, 19-67 years). More than two thirds of participants interviewed in both stores were employees (human, 73% and veterinary, 74%), and the remainder were store owners. Significantly more human pharmacists (90%) than veterinary pharmacists (57%) had undergone some form of clinical training ($P = 0.01$, Fisher exact test). Additionally, for participants who underwent clinical training, professional development programmes/trainings aimed at continuing education in AMR were an important source of information on antibiotic stewardship (human pharmacists, 50%; and veterinary pharmacists, 41%).

Antibiotics sold and sale dynamics

A total of 15 antibiotic classes were available in either or both human and veterinary drug stores (**Figure 2**). Two thirds of the antibiotic classes (10/15) were found in both human and veterinary drug stores while five classes (metronidazole, amphenicols, lincosamides, glycopeptides and carbapenems) were only found in human drug stores. Of the ten overlapping antibiotic classes, beta lactam/penicillin, tetracycline, sulfonamide, and macrolide antibiotic classes were found in more than 78% of both types of drug stores. Of note, carbapenems, third and fourth generation-cephalosporins and glycopeptides – antibiotics restricted to clinical use – were found in 15%, 4% and 3% of human drug stores respectively. Overall, human drug stores had a broader range of antibiotics available for sale when compared to veterinary stores ($P < 0.01$, Mann-Whitney U test) (**Figure 2**).

Penicillins, metronidazole, fluoroquinolones, and first and second-generation cephalosporins were reported as being amongst the four most commonly sold antibiotic classes by the human drug stores in 93%, 65%, 63%, and 43% of the stores respectively. However, among the veterinary drug stores, tetracyclines, sulfonamides, penicillins and macrolides were reported to be amongst the four most commonly sold antibiotic classes in 79%, 74%, 58%, and 47% of the stores respectively. Tetracyclines and sulfonamides were reported to be amongst the four most commonly purchased antibiotic classes by poultry farmers in 79% and 90% of the veterinary drug stores respectively. The antibiotic colistin was described as being commonly purchased by poultry farmers in 16% of the drugs stores. Sulfonamides, tetracyclines and penicillins were reported to be amongst the four most commonly purchased antibiotic classes by dairy farmers in 63%, 47% and 52% of drug stores respectively. In 11% of the veterinary drug stores, dairy farmers reportedly purchased first and second-generation cephalosporin intra-mammary tubes to treat mastitis cases. The antibiotics reported to be amongst the four most commonly purchased antibiotic classes by pig farmers were penicillins, macrolides and sulphonamides in 37%, 16% and 11% of the veterinary drug stores respectively (**Figure 3**).

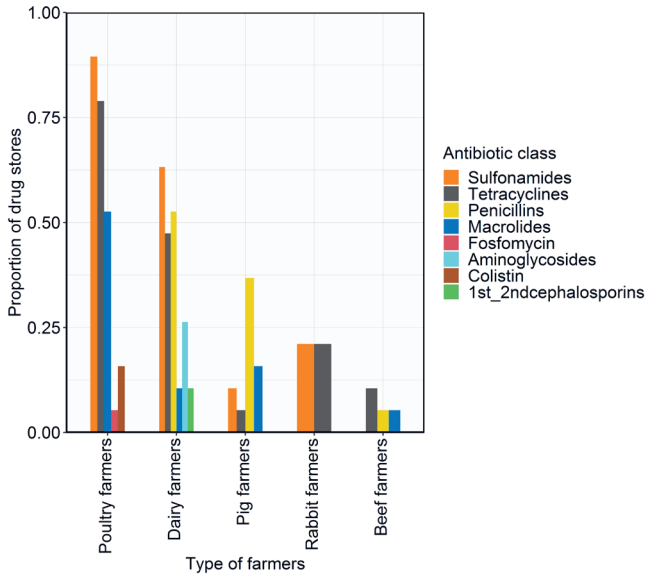

Figure 3. Proportion of drug stores reporting the most commonly purchased antibiotics by different types of farmers based on the primary animal on the farm.

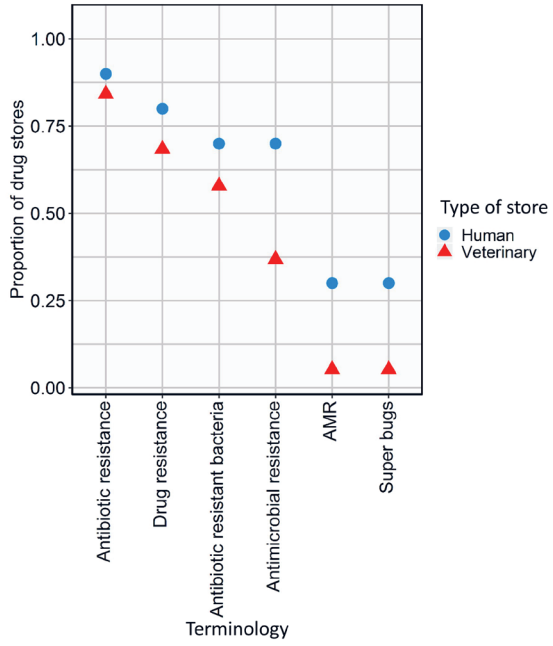

Figure 4. Terms used to describe antimicrobial resistance.

Fifty eight percent of human and 42% of veterinary drug stores reported a rise in antibiotic sales compared to the same period a year earlier. Increased customer demand for antibiotics was believed to be the main driver by 80% and 60% of human and veterinary pharmacists respectively. Wholesale operations (defined as companies that buy drugs in bulk and sell them in smaller quantities to drug stores) were reported as the main provider of antibiotics to human drug stores (78%). On the other hand, distribution companies (defined as corporations that purchase drugs from pharmaceutical companies, store and subsequently distribute to drug stores) were reported as the main provider of antibiotics to veterinary drug stores (58%).

Antibiotic customer characteristics

The average daily number of customers purchasing antibiotics was not significantly different ($P=0.20$; Mann-Whitney U test) between human drug stores (25 customers, range 2-130) and veterinary drug stores (14 customers, range 2-113).

Antibiotics were reportedly prescribed frequently for respiratory tract infections, gastro-intestinal infections, and sore throat in 83%, 65% and 58% of human drug stores respectively. Additional prescriptions were linked to fever, body aches, and skin wounds in 38%, 35% and 13% of human drug stores respectively.

Poultry farmers and veterinary para-professionals were the most frequent customers of antibiotics, being reported as customers in 95% and 74% of the veterinary drug stores respectively. Other customers for antibiotics included: dairy farmers, veterinarians, pig farmers, rabbit famers, and beef farmers in 63%, 58%, 47%, 37%, and 11% of stores.

Knowledge of antimicrobial resistance

More than two-thirds of the respondents in both stores types were aware of the terms "antibiotic resistance" and "drug resistance". By contrast, fewer than half of respondents had heard of the terms "AMR" and "super bugs" (**Figure 4**).

More than three quarters of the respondents in both store types agreed that the prevalence of drug resistant infections was increasing, and if left unchecked routine medical and surgical procedures would become a much riskier proposition. Likewise, more than 79% in both types of stores recognised that AMR is a problem, and has the potential to affect any country and anyone, including them and/or their families. However, most respondents (>80%) believed that AMR occurs when their body becomes resistant to antibiotics rather than the bacteria themselves that develop resistance. Similarly, 40% and 53% of human and veterinary respondents respectively suggested that AMR is only a problem for regular consumers of antibiotics. More than half (52%) of the respondents interviewed responded neutrally or disagreed with the statement that antibiotic resistant bacteria could be spread from person to person (Table S3 and Figure S2 in **Online Supplementary Document**).

Association between sociodemographic factors and knowledge on AMR

Cronbach's alpha coefficient for the ten "knowledge statements" was 0.74, suggesting an acceptable level of internal consistency and a potential underlying latent construct (Table S2 and Figure S1 in **Online**

Supplementary Document). The knowledge score (knowledge about AMR) of the respondents had significant positive association with medical/veterinary training ($P=0.02$), meaning respondents with clinical training had a higher knowledge about AMR than those who had not undergone such training. The level of knowledge about AMR did not differ by store type, education level or range of antibiotics available in the store ($P>0.05$) (**Table 2**).

Table 2. Results of a multivariable regression examining the influence of sociodemographic factors on knowledge about AMR in a sample of 40 and 19 human and veterinary drug stores respectively.

Variable	Estimate	Standard error	x^2	df	*P*-value
Veterinary drug store	0.07	0.02	0.3	1	0.59
Range of antibiotics	-0.01	0.02	0.31	1	0.58
Clinical/veterinary training	0.3	0.14	4.86	1	0.02
High education level	0.04	0.12	0.13	1	0.72

Knowledge and views on potential solutions to AMR

More than 80% of respondents in both store types agreed that people should use antibiotics only when prescribed by a medical practitioner. Also, more than two thirds of respondents in both store types agreed that reducing antibiotic use in food animals could help address the problem of antibiotic resistance. In both store types, respondents agreed on the need for governments and pharmaceutical companies to invest in research and development of new antibiotics. More than 84% of all respondents agreed that everyone should use antibiotics prudently, but more than 73% of respondents thought that medical experts would solve the problem of antibiotic resistance. Hand washing and vaccination of children against infections were supported by more than 94% of respondents in both store types. However, 38% and 26% of respondents in human and veterinary drug stores agreed that there was not much they could do to stop antibiotic resistance (Table S4 and Figure S3 in **Online Supplementary Document**).

Antibiotic prescribing practices

Fifty-two per-cent (21/40) of the human drug stores reported that they sold antibiotics without a prescription while all veterinary drug stores sold antibiotics without a prescription. Multivariable logistic regression analysis revealed prescribing practices did not vary significantly by clinical training, store type, range of antibiotics sold and/or education level ($P>0.05$) (Table S5 in the **Online Supplementary Document**).

Across both human and veterinary drug stores, the most important factor for prescribing antibiotics was indication of use – based on symptoms – (in >75% of the stores), followed by price of the antibiotic (in >50% of the stores). Of note, 28% and 31% of human and veterinary pharmacists respectively considered customer preference as an important factor when prescribing an antibiotic.

DISCUSSION

In this study, we aimed to investigate the patterns of antibiotic sales in humans and animals in a large and rapidly developing city in a LMIC: Nairobi, Kenya. We also evaluated the level of awareness and common behaviours related to antibiotic use and AMR amongst human and veterinary pharmacists. Our study was based on gathering antibiotic sales data from human and veterinary drug stores across the city, where sales data were interpreted as representing antibiotic usage.

Our study shows considerable overlap in the antibiotic classes (10/15) sold for human and animal use in urban Nairobi, with marked variations in the sale of some antibiotic classes such as cephalosporins and fluoroquinolones – mostly found in human drug stores. This overlap in antibiotic classes, including of critically important antimicrobials [24], highlights the need for prudent use of all antimicrobials and continued monitoring and surveillance of antimicrobial usage in LMIC urban settings [25].

The most common symptoms prompting antibiotic purchase in humans were similar to those reported in other studies, respiratory tract infections and diarrhoeal disease [7,26,27]. Broad-spectrum beta lactams, fluoroquinolones, first and second-generation cephalosporins and metronidazole were the most commonly sold/bought antibiotics in human drug stores. This finding is consistent with antibiotic prescription in the community in previous Kenyan studies [27,28], in other low income countries such as Uganda [29], Tanzania [30], India [31] and in high income countries such as United Kingdom [32] and the USA [33].

Our finding that WHO-classified highest priority critically important antibiotic classes such as carbapenems, third and fourth generation cephalosporins, and glycopeptides were sold over the counter and potentially without prescription in human drugs stores is of public health concern.

In the current study, tetracyclines, sulphonamides, penicillins, and macrolides were the most commonly purchased veterinary antibiotics and poultry farmers were the major consumers of antibiotics. Further, our findings indicate that colistin – a drug considered of last resort in human medicine [34] – was an antibiotic of choice amongst poultry farmers in 16% of veterinary drug stores, as has been found in previous studies in other parts of the world [35-37]. Urban livestock are increasingly important, particularly among the low and middle income population bracket in most low resource urban settings [38,39], and antibiotic usage is a low-cost alternative for comprehensive hygiene and biosafety measures [40].

Knowledge about antimicrobial resistance among pharmacists has only been studied to a limited extent in LMICs [41]. Consistent with a recent multi-country survey by the World Health Organization [12] our survey found that, whilst the majority of the pharmacists have an understanding of the problem of antibiotic resistance and the effect(s) on public health, they do not fully understand how AMR develops and spreads. Encouragingly, the majority of respondents (>80%) identified several behaviours that can help reduce AMR burden; such as handwashing, antibiotic stewardship by both doctors and the public, and ensuring children's vaccinations are up-to-date. However, considering their key role in antibiotic stewardship, the finding that 38% and 26% of human and veterinary pharmacists agreed there was little they could do to stop AMR highlights the need for enhanced involvement of pharmacists in antibiotic stewardship programs.

Whilst the majority of the pharmacists we interviewed have an understanding of the threat posed by AMR to public health, our data highlight the poor quality of community pharmacy practice, most notably the dispensing of antibiotics without prescriptions and the inclusion of customer preference as an important factor when selling antibiotics. Antibiotics were dispensed without prescription in 53% and 100% of the human and veterinary drug stores respectively; a finding consistent with similar studies in Tanzania (92.3%) [42], Serbia (47.2%) [43], Ghana (70%) [44], and broadly across the developing world (19%-100%) [25]. By contrast, a recent study conducted in community pharmacies in Nairobi reported low sale of antibiotics without prescription [45]. Part of this difference, however, may be related to the fact that in that study, information was based on just three pharmacies hence not generalizable across the city. In our study, whilst clinical training significantly influenced knowledge on issues related to antibiotic use and AMR, prescribing practices did not change with levels of clinical training. Considering the complexity of factors contributing to antibiotic prescribing, including the public's demand for antibiotics, behavioural and policy interventions could be explored [46]. Because many members of the public in most LMICs bypass health care facilities and veterinarians in favour of seeking medication at pharmacies, policy makers could consider expanding the role of pharmacists in antibiotic stewardship initiatives [47,48].

Restating the particular relevance of training to antibiotic stewardship measures, the role of enhanced training in antimicrobial prescribing and AMR has been identified in surveys of both medical personnel and the public, both in Kenya and globally [49]. Results from a recent survey indicate that only 14.1% of clinicians in a national referral and teaching hospital in Kenya had received more than four lectures on antimicrobial stewardship and AMR as part of their medical training [50]. To address this challenge, antibiotic stewardship needs to be integrated in the undergraduate veterinary/medical curriculums and continuing medical/veterinary education programs.

Similar to other studies [51,52], our findings indicate greater familiarity amongst human and veterinary pharmacists with 'antibiotic resistance' and 'drug resistance' terminologies, and minimal familiarity with 'AMR' and 'superbugs'. This indicates that public health initiatives on antibiotic stewardship and/or antimicrobial resistance initiatives need to take an evidence-based approach in designing effective communication strategies [51,53].

This is the first study designed to capture the overlapping patterns of antibiotic sales in humans and livestock in a developing city via an epidemiologically-structured approach. A variety of approaches are available for assessing patterns of antibiotic use in humans and animals [54]. Considering that pharmacies are the primary level of outpatient/veterinary care (consultation, diagnosis, and prescription of antibiotics) for many urban dwellers in Nairobi, focusing on them provides important insights into the probable antibiotic usage patterns at the consumer level. Future research would benefit from conducting longitudinal surveys of antimicrobial use in health care facilities and the community to better assess trends over time. While, we acknowledge that our study used a relatively small sample size (19 and 40 veterinary and hu-

man drug stores respectively), we did not find much heterogeneity in the results obtained. It is important to highlight that although extrapolating antimicrobial consumption from sales data are not ideal, and care will be required when interpreting our results, various other studies have shown that relying on sales data are of direct relevance for initiatives aimed at monitoring global and national antimicrobial patterns [55,56]. Although this study focused on pharmacists in urban Nairobi, these results are likely to be relevant to many other developing cities across the world with large income disparity and where livestock are commonly kept in close contact with humans.

CONCLUSIONS

Monitoring and surveillance of antibiotic use in LMICs is challenging, but vital, as it provides valuable information for public health policy. Our study shows that at the retail level in urban Nairobi, there is a considerable overlap between antibiotic classes available for use in both human and veterinary medicine. Whilst the majority of human and veterinary pharmacists showed high knowledge about antibiotic use and antimicrobial resistance, inappropriate prescribing practices were noted, highlighting the need for continued education to the pharmacists and the public, about prudent antibiotic prescribing and use. Although further research is necessary to understand the drivers of antibiotic consumption in both populations, it is clear that interventions are urgently required to improve prescribing practices across the pharmacists.

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Authorship contributions: EMF, MW, DM and MJW. conceived the study. DM collected field data, performed data analysis and drafted the manuscript. JB, MKK, PM and AS were involved in the design of data collection protocols. RR and DO collected field data. JMH, BvB, and MCT were involved in data analysis and contributed intellectually to the manuscript. MW, EMF, ABP and MJW contributed intellectually to the manuscript. All authors provided comments on the manuscript and gave final approval for publication. The views expressed are those of the authors and are not necessarily those of the CGIAR or of other institutions to which the authors are affiliated.

Competing interests: The authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author), and declare no conflict of interest.

Additional material
Online Supplementary Document

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Clinically relevant antimicrobial resistance at the wildlife–livestock–human interface in Nairobi: an epidemiological study

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Summary

Background Antimicrobial resistance is one of the great challenges facing global health security in the modern era. Wildlife, particularly those that use urban environments, are an important but understudied component of epidemiology of antimicrobial resistance. We investigated antimicrobial resistance overlap between sympatric wildlife, humans, livestock, and their shared environment across the developing city of Nairobi, Kenya. We use these data to examine the role of urban wildlife in the spread of clinically relevant antimicrobial resistance.

Methods 99 households across Nairobi were randomly selected on the basis of socioeconomic stratification. A detailed survey was administered to household occupants, and samples (n=2102) were collected from the faeces of 75 wildlife species inhabiting household compounds (ie, the household and its perimeter; n=849), 13 livestock species (n=656), and humans (n=333), and from the external environment (n=288). *Escherichia coli*, our sentinel organism, was cultured and a single isolate from each sample tested for sensitivity to 13 antibiotics. Diversity of antimicrobial resistant phenotypes was compared between urban wildlife, humans, livestock, and the environment, to investigate whether wildlife are a net source for antimicrobial resistance in Nairobi. Generalised linear mixed models were used to determine whether the prevalence of antimicrobial resistant phenotypes and multidrug-resistant *E coli* carriage in urban wildlife is linked to variation in ecological traits, such as foraging behaviour, and to determine household-level risk factors for sharing of antimicrobial resistance between humans, wildlife, and livestock.

Findings *E coli* were isolated from 485 samples collected from wildlife between Sept 6,2015, and Sept 28, 2016. Wildlife carried a low prevalence of *E coli* isolates susceptible to all antibiotics tested (45 [9%] of 485 samples) and a high prevalence of clinically relevant multidrug resistance (252 [52%] of 485 samples), which varied between taxa and by foraging traits. Multiple isolates were resistant to one agent from at least seven antimicrobial classes tested for, and a single isolate was resistant to all antibiotics tested for in the study. The phenotypic diversity of antimicrobial-resistant *E coli* in wildlife was lower than in livestock, humans, and the environment. Within household compounds, statistical models identified two interfaces for exchange of antimicrobial resistance: between both rodents, humans and their rubbish, and seed-eating birds, humans and their rubbish; and between seed-eating birds, cattle, and bovine manure.

Interpretation Urban wildlife carry a high burden of clinically relevant antimicrobial-resistant *E coli* in Nairobi, exhibiting resistance to drugs considered crucial for human medicine by WHO. Identifiable traits of the wildlife contribute to this exposure; however, compared with humans, livestock, and the environment, low phenotypic diversity in wildlife is consistent with the hypothesis that wildlife are a net sink rather than source of clinically relevant resistance. Wildlife that interact closely with humans, livestock, and both human and livestock waste within households, are exposed to more antimicrobial resistant phenotypes, and could therefore act as conduits for the dissemination of clinically relevant antimicrobial resistance to the wider environment. These results provide novel insight into the broader epidemiology of antimicrobial resistance in complex urban environments, characteristic of lower-middle-income countries.

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Introduction

Antimicrobial resistance in bacteria is one of the great challenges facing global health security in the modern era, and will ultimately limit our capacity to treat microbial infections. The repercussions for human and domestic animal health are severe; as infections become more difficult and costly to treat, morbidity and mortality will increase, and the extra burden placed on health services and livestock production will have considerable economic consequences.[1]

The two most probable sources of clinically relevant antimicrobial resistance are the exposure of pathogens to antibiotic use in humans and in livestock.[2] Little is known about the ecology of antimicrobial resistance

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Research in context

Evidence before this study

We searched PubMed for the terms "wildlife", "antimicrobial resistance", and "urban", with no date limits set and language limited to English. Earlier studies described antimicrobial resistance in select species of urban wildlife, and a single study compared differences in prevalence between wildlife and livestock (cattle, on UK dairy farms). No published studies examined the presence of antimicrobial resistance across diverse urban wildlife taxa inhabiting the same urban environment, and no studies compared antimicrobial resistance in sympatric wildlife, livestock, and human populations.

Added value of this study

Ecological and epidemiological approaches were applied to provide, to our knowledge, the first epidemiologically structured comparative analysis of phenotypic antimicrobial resistance characterisation in sympatric wildlife, livestock, humans, and the environment in an urban setting, and the most comprehensive analysis of urban wildlife-borne antimicrobial-resistant phenotypes so far. Because this study was done on a city-wide scale, it allowed us to evaluate carriage of clinically relevant antimicrobial resistance in urban wildlife across Nairobi, and relate this to antimicrobial resistance in the broader urban epidemiological system. We present several important findings, showing that, although urban wildlife carry high burdens of clinically relevant antimicrobial resistance, phenotypic diversity is lower than in humans, livestock, or the external environment. Wildlife that associate closely with livestock, humans, and both livestock and human waste are exposed to higher levels of antimicrobial-resistant phenotypes than wildlife that do not associate as closely with livestock and human waste, and could thus act as conduits for dissemination to the wider environment. Our findings emphasise the importance of understanding ecological flows of antimicrobial resistance within complex urban systems, to inform strategies aimed at limiting human exposure to multidrug-resistant bacteria.

Implications of all the available evidence

The results of this study and previous studies suggest that through anthropogenic exposure, wildlife have a taxa-specific role in the acquisition and dissemination of clinically relevant antimicrobial resistance across urban landscapes, and have the potential to disseminate antimicrobial resistance from urban areas to broader ecosystems. Similarly scaled future studies done in a variety of urban settings would permit examination of context-specific differences in wildlife antimicrobial resistance carriage and exposure. More broadly, contamination of urban environments with antimicrobial resistance is a serious issue, and future studies should focus on identifying antimicrobial resistant flow through urban ecological systems, and relating this to coresistance and crossresistance to other environmental pollutants (such as heavy metals). Such evidence could be used to develop cost-effective surveillance for urban ecological systems, and to inform interventions that are aimed at limiting environmental contamination with pollutants of public health significance. Ultimately, this work forms part of a broader strategy to understand the epidemiology of antimicrobial resistance across developing urban landscapes.

outside human and livestock hosts, but it is increasingly clear that focusing only on these compartments of the transmission system will result in an incomplete epidemiological picture of resistance.[3] Bacterial populations in aquatic and soil habitats are enormously diverse, and have crucial roles in nitrogen cycling, carbon sequestration, and the stability of aquatic ecosystems.[4] These bacteria also act as reservoirs of naturally occurring bacterial resistance, the burden of which is exacerbated by flows of resistance elements and other chemicals (such as heavy metals) from livestock and human waste, which can coselect for drug resistance.[5] Resulting changes to microbial diversity could lead to damaging effects on terrestrial and aquatic ecosystems, such as nitrification and mobilisation of heavy metals.[6,7]

Wildlife exist across multiple trophic levels, and are therefore well placed to accumulate and disperse resistance determinants within ecosystems. Ecological traits, such as habitat, feeding preferences, and ranging behaviour could determine the exposure of wildlife species to antimicrobial resistance, and how widely it is dispersed in the environment.[8,9] The presence of diverse bacterial resistance profiles in wildlife inhabiting pristine environments also shows the complexity of naturally occurring antimicrobial-resistant communities in the gut of free-ranging vertebrates, for which environmental acquisition probably has an important role.[10,11] As land-use changes reduce the availability of natural habitats, wildlife species are forced to seek alternative sources of food and shelter, bringing them into closer association with humans, livestock, and their waste, and increasing the potential for transfer of antimicrobial resistance between them.[3,12]

In lower-middle-income countries, urban environments act as hotspots for interactions between humans, animals, and their shared environment. The focus of this study is on the informal keeping of livestock by households in Nairobi, Kenya, as a potentially high-risk urban interface for antimicrobial-resistant transmission between wildlife, humans, livestock, and the environment. Livestock are frequently kept within household perimeters in low-income country urban centres, where differing levels of waste management could cause variation in environmental dispersal of determinants of, and exposure of wildlife to, antimicrobial resistance.[12] Being ubiquitous in vertebrates and the environment, *Escherichia coli* is frequently targeted in studies of antimicrobial resistance, and is an ideal sentinel bacteria

for the study of the dispersal of antimicrobial resistance across diverse vertebrate host species and the environment.[8]

Using *E coli* antimicrobial-resistance phenotypes collected from households across Nairobi, we explored the role of urban wildlife in the epidemiology of antimicrobial resistance. In considering antimicrobial resistance as defined by clinically significant human treatment breakpoints and to antibiotics of importance in human medicine, the true clinical relevance of antimicrobial resistance in urban wildlife is examined.[13] Wildlife, which are not treated with antibiotics, might be a net recipient (or sink) of antimicrobial resistance in urban environments, while acting as an effective conduit of antimicrobial resistance between other parts of the system. These hypotheses are tested by using statistical models to compare the carriage of clinically relevant antimicrobial resistance between epidemiological compartments (ie, wildlife, humans, livestock, and the environment). To further understand the determinants of exposure of wildlife to antimicrobial resistance, variation in host taxon and functional ecology (eg, foraging traits) are related to carriage of multidrug-resistant *E coli*, and antibiogram length in wildlife across the city. At a finer scale, epidemiological models are used to investigate risk factors for exchange of antimicrobial resistance between sympatric wildlife, humans, and livestock, thus shedding light on pathways of antimicrobial resistance transfer at household interfaces.

Methods

Study design

Faecal samples (n=2081) from 75 wildlife species (birds and mammals [n=794], appendix), 13 livestock species (n=677), humans (n=333), and samples from the external environmental (n=277) were collected from households across Nairobi that were participating in the UrbanZoo 99-household project between Sept 6, 2015, and Sept 28, 2016.[14] An additional 24 faecal samples were collected from birds and rodents in abattoirs across the city. Our study design is explained in detail in the appendix; briefly, Nairobi was split into administrative units, and 33 were chosen on the basis of a socioeconomic stratification. Three households were randomly selected in each sublocation to obtain two livestock-keeping and one non-livestock-keeping household (a total of 99 households), with the aim of maximising the spatial distribution and diversity of livestock-keeping practices captured within the sampling frame. Wildlife samples were also obtained from an additional household, where the occupants declined to submit human samples or questionnaire data. As such, 100 households were included in analyses in which isolates from wildlife were considered alone. Households in each sublocation had to meet strict inclusion criteria of keeping small ruminants or poultry, large ruminants or pigs, or no livestock within the household perimeter. Abattoirs in Nairobi were selected and sampled in a separate value chain study done as part of the wider UrbanZoo project.[15] Wildlife samples were obtained by a range of taxon-specific trapping methods, which are described in the appendix, along with protocols for collection of human, livestock, and environmental samples. Questionnaires detailing household composition and socioeconomic data, and livestock ownership and management, were administered at each household (appendix). Household occupants who provided samples and answered questionnaires provided written consent.

The collection of data adhered to the legal requirements of the country in which the research was conducted. Wildlife were trapped under approval of an International Livestock Research Institute (ILRI) Institutional Animal Care and Use Protocol (IACUC; 2015.12), and permits obtained from the National Museums of Kenya and Kenya Wildlife Service. Livestock samples were obtained under approval of ILRI IACUC (2015.18). Human samples and questionnaire data were collected under approval of ILRI Institutional Research Ethics Committee approval (2015-09).

Microbiological testing

All rectal swabs and fresh faecal samples were placed in Amies transport media, and transported on ice to one of two laboratories (Kenya Medical Research Institute or University of Nairobi [UoN]). Boot socks (on which surface material from livestock pens and the external environment were collected) and modified Moore swabs were transported in saline-filled polythene bags, and water samples were transported in conical tubes, all on ice. Samples were enriched in buffered peptone water for 24 h, and then plated onto eosin methylene blue agar (EMBA) and incubated for 24 h at 37°C. Subsequently, five colonies were selected and subcultured on EMBA, before being further subcultured on Müller-Hinton agar and stored at –20°C in cryovials. A single colony was picked at random from the plate for each original sample (ie, an isolate) and biochemical tests (triple sugar iron agar, Simmon's citrate agar, and motility-indole-lysine media) were used for presumptive identification of *E coli*. A single colony was picked from each avian or bat pooled faecal sample.

See Online for appendix

All isolates were revived and inoculated onto Müller-Hinton plates before antimicrobial susceptibility testing. Isolates were tested for susceptibility to ampicillin (10 µg), amoxicillin–clavulanic acid (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), streptomycin (25 µg), sulfamethoxazole (30 µg), tetracycline (30 µg), and trimethoprim (2·5 µg) using the disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines.[13] Antibiotics included those frequently used in both veterinary and human medicine in Kenya.[16] Clinical and Laboratory Standards Institute

guidelines were also used to determine human breakpoints for classifying isolates as sensitive, intermediate, or resistant to the drug.[13] Following previous studies, intermediate strains were deemed to be moving towards resistance, and thus considered resistant on an evolutionary basis.[17,18] All protocols were standardised between laboratories, and between-laboratory quality control was done at regular intervals. Multidrug-resistant *E coli* was defined as "non-susceptibility to at least one agent in three or more antimicrobial classes"[17] (appendix). Wildlife isolates were also assessed for high levels of multidrug resistance (non-susceptibility to at least seven antimicrobial classes tested) and resistance to all antibiotics tested for in this study. An antibiogram was defined as the combination of antibiotics to which an isolate was resistant, and thus antibiogram length was defined as the number of antibiotics to which an isolate was phenotypically resistant.

Statistical analysis

All statistical analyses were done using R, version 3.3.2. Spatial structure in the dataset was represented using distance-based Moran's eigenvector maps—a powerful multivariate approach to model spatial structure in a response variable, which can be partitioned at broad, medium, and fine spatial scales.[19,20] Further details of how we dealt with missing data, data exploration, and statistical models (distributions, choice of fixed and random effects, implementation, and model selection procedures) are given in the appendix.

To test the hypothesis that urban wildlife are a net source or sink of antimicrobial resistance in Nairobi when compared with humans, livestock, and the environment, epidemiological and ecological statistical modelling approaches were applied. Prevalence of resistance to 13 antibiotics was compared between all four epidemiological compartments (ie, wildlife, human, livestock, environment) in a Bayesian analysis framework, using Markov Chain Monte Carlo methods.[21,22] Generalised linear mixed effects models (GLMMs) with binomial (log-link function) and Poisson distributions were used to test whether multidrug-resistant *E coli* carriage and antibiogram length differed between compartments, and how this varied spatially across the city. To assess how antibiogram diversity was distributed across compartments, antibiogram diversity was compared using four ecological measures of diversity related to Rényi's measures of generalised entropy.[23] Methods adapted from community ecology were used to extend the comparison of phenotypic diversity between compartments by estimating the number of undetected antibiograms. Chao2, ICE, and Jack-knife incidence-based statistical methods were used to estimate the minimum total antibiogram richness in each compartment from the data, by looking at frequencies of phenotype occurrence in collections of individuals. To consider the implications for surveillance, methods from Chao and colleagues[24] were followed to estimate the sampling effort required to detect a given proportion of the total antibiograms estimated for each compartment. Our approach is described in full in the appendix.

A Bayesian analysis framework, as described earlier, was used to estimate and compare prevalence of resistance to 13 antibiotics between wildlife taxa. Ecological traits considered potentially important factors for exposure of wildlife to antimicrobial resistance were modelled against multidrug-resistant *E coli* carriage and antibiogram length in wildlife in binomial and Poisson GLMMs, respectively. Separate binomial GLMMs were developed to investigate fine-scale household-level risk factors for the likelihood of multidrug-resistant *E coli* carriage in select urban wildlife with synanthropic traits (ie, rodents and seed-eating birds). Risk factors were sourced from a set of anthropogenic and ecological covariates capturing antimicrobial-resistant *E coli* carriage in humans and livestock, livestock-keeping practices, land use within households, and ranging behaviour of wildlife. All anthropogenic and ecological variables were derived from metadata collected within households, and published sources (appendix). The laboratory in which samples were tested was included as a confounding factor in these models.

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, or interpretation. JMH and EMF always had full access to the data in the study, and had final responsibility for the decision to submit for publication.

Results

Samples were collected from 547 individual birds, nine avian populations (31 pooled samples across nine populations), 167 rodents, 44 individual bats, five bat populations (20 pooled samples across nine populations), five carnivores, and four primates across 100 households, as well as from 11 abattoirs in Nairobi, between Sept 6, 2015, and Sept 28, 2016. Antimicrobial susceptibility tests were done on a single *E coli* isolate cultured from 282 (52%) of 547 birds, 20 (65%) of 31 avian populations, 155 (93%) of 167 rodents, 22 (50%) of 44 bats, six (22%) of 27 bat populations, three (60%) of five carnivores, and four (100%) of four primates. Because of low sample numbers, primates and carnivores were not included in further statistical analysis, and each pooled population sample was considered as coming from an individual bird or bat for the purposes of all further analysis. *E coli* was isolated from, and antimicrobial susceptibility tests done on, 638 livestock, 321 human, and 256 environmental samples. 252 (52%) of 485 samples from wildlife sampled in Nairobi carried multidrug-resistant *E coli*; eight (2%) of 485 wildlife isolates (all originating from birds) carried *E coli* resistant

	Estimate	SE	Z score	p value
Model: MDR carriage in all isolates				
Intercept	0·030	0·102	0·296	0·77
Environment	0·322	0·165	1·954	0·051
Human	0·662	0·155	4·273	<0·0001
Livestock	0·284	0·128	2·222	0·026
MEM1	0·148	0·067	2·225	0·026
MEM2	–0·118	0·066	–1·781	0·075
MEM5	–0·130	0·063	–2·072	0·038
Model: antibiogram length of all isolates				
Intercept	1·095	0·031	35·8	<0·0001
Environment	0·107	0·044	2·42	0·015
Human	0·199	0·040	4·94	<0·0001
Livestock	0·070	0·036	1·96	0·049
MEM1	0·070	0·021	3·31	0·00093
MEM2	–0·042	0·02	–2·04	0·041
MEM5	–0·049	0·019	–2·60	0·0095

SE=standard error. MDR=multidrug resistance. MEM1, MEM2, and MEM5 indicate the spatial scales across which variation in MDR carriage or antibiogram length occurs.

Table 1: **Estimated regression parameters, SEs, Z scores, and p values for generalised linear mixed models**

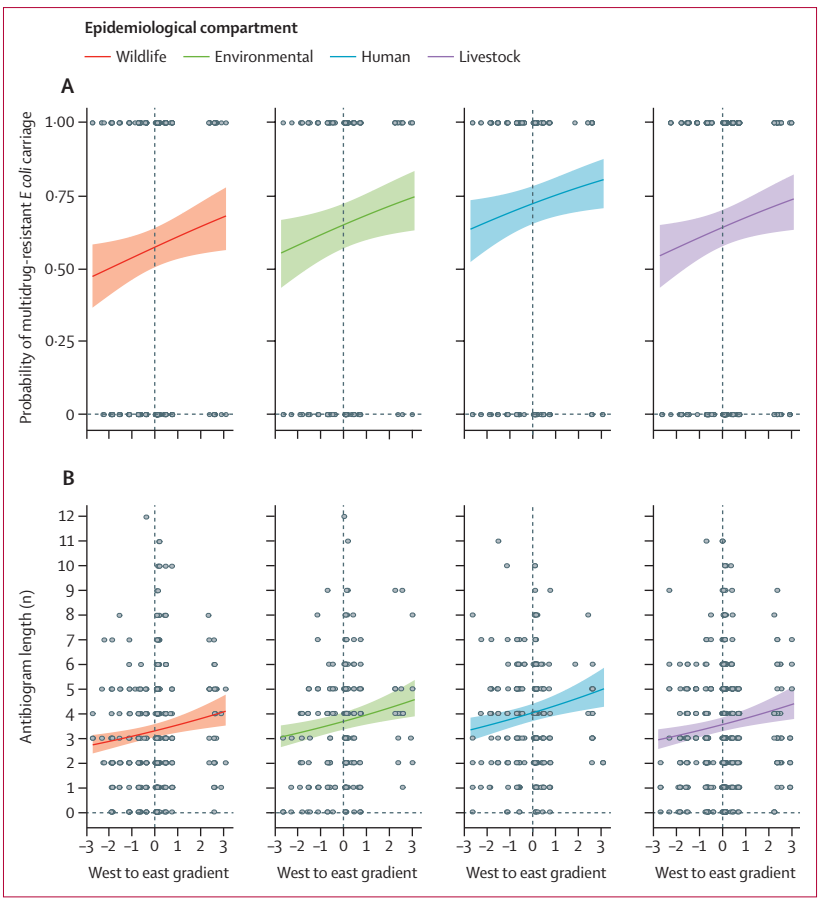

Figure 1: **Variation in probability of multidrug resistant *Escherichia coli* carriage (A) and antibiogram length (B) in different epidemiological compartments along a west to east gradient across Nairobi**
Coloured shading represent 95% CI.

to agents belonging to at least seven of the antimicrobial classes tested; and *E coli* isolated from a single avian sample was resistant to all antimicrobials tested.

Prevalence of antimicrobial-resistant *E coli* in wildlife was significantly lower than at least one epidemiological compartment for six of the antibiotics tested (ampicillin [human], cefepime [livestock], cefotaxime [livestock], streptomycin [human], tetracycline [human, livestock, environment], and trimethoprim [human]), and not significantly higher than other compartments for any of the 13 antibiotics tested (appendix). Wildlife were less likely to carry multidrug-resistant *E coli* than humans and livestock (β=0·662, 95% CI 0·36 to 0·97, p<0·0001; β=0·284, 95% CI 0·03 to 0·53, p=0·026), and had shorter antibiogram lengths than all other compartments (marginal R^2 0·028; table 1). Five distance-based Moran's eigenvector maps were associated with multidrug-resistant *E coli* carriage and antibiogram length of isolates, and were thus included as covariates in the GLMMs. Both models showed broad-scale spatial relationships for antimicrobial resistance carriage across the city; the probability of multidrug-resistant *E coli* carriage in all epidemiological compartments increased along a west to east gradient (MEM1; β=0·15, 95% CI 0·02 to 0·28, p=0·026; marginal R^2 0·028; figure 1), whereas antibiogram lengths decreased from eastern to western Nairobi (MEM1) and increased from northern to southern Nairobi (MEM2; β=0·07, 95% CI 0·03 to 0·11, p=0·00093; β=–0·04, 95% CI –0·08 to 0, p=0·041; marginal R^2 0·028, 0·043; figure 1).

Population-diversity measures of resistance indicated that wildlife had less diverse antibiograms than other compartments. *E coli* isolated from wildlife had a lower expected antibiogram diversity than all other compartments as measured by three of the four D_α diversity indices calculated (Shannon entropy , Simpson diversity, and Berger-Parker; appendix). When compared across all compartments, the range of median α values was significantly lower in wildlife than all other compartments (wildlife:environmental p=0·0079; wildlife:livestock p=0·002; wildlife:human p=0·00021). Asymptotic estimates of minimum total antibiogram richness in wildlife were 273 (95% CI 245–300) unique antibiograms, most of which could be detected if an additional 8848 samples were collected (figure 2; appendix). This richness estimate is lower than estimates for the environment (350, 95% CI 305–395) and livestock (416, 378–454), but higher than the estimate for humans (185, 165–205). Unlike the human compartment, where an asymptote was reached at 270 samples, wildlife and livestock estimates were only beginning to reach an asymptote at the sampling extremes achieved in this study. None of the statistical estimators reached an asymptote for environment, suggesting that the rate of discovery of new antibiograms in this compartment was

still high, and minimum richness estimates could therefore be considerably higher than 350.

When split into taxonomic groups, prevalence of *E coli* isolates susceptible to all antibiotics tested was 45 (9%) of 485 samples across all wildlife, 26 (9%) of 282 birds, two (10%) of 20 avian populations, 13 (8%) of 155 rodents, and four (14%) of 28 bats. Bayesian models showed that prevalence of resistance to streptomycin, tetracycline and trimethoprim varied significantly between wildlife when stratified by taxonomic or functional groups (appendix). Birds belonging to the orders Pelecaniformes and Ciconiiformes were more likely to carry *E coli* resistant to ceftazidime (odds ratio 7·9, 95% CI 1·7–28·5; p=0·0033), and had significantly longer antibiograms than other species of wildlife (p=0·04).

Multidrug-resistant *E coli* carriage varied by taxonomic functional groups, and along an east to west gradient across Nairobi (marginal R^2 0·08; figure 3). Frugivorous bats and seed-eating, omnivorous, and scavenging birds were significantly more likely to carry multidrug-resistant *E coli* than frugivorous birds, and the probability of carrying multidrug-resistant *E coli* increased significantly from west to east Nairobi (appendix). *E coli* antimicrobial resistant antibiograms were longer in birds than rodents (β=–0·16, 95% CI –0·29 to –0·03, p=0·016), and antibiogram length showed spatial correlation across multiple scales of the city (broad-scale [east to west; MEM1], medium-scale [MEM8, 10, 19], and fine-scale [MEM25, MEM27] resolutions; marginal R^2 0·13; appendix). Wildlife-borne *E coli* processed at UoN laboratories had significantly longer antibiograms. The effects of laboratory were only present in a single model, and all reasonable efforts were taken to ensure that

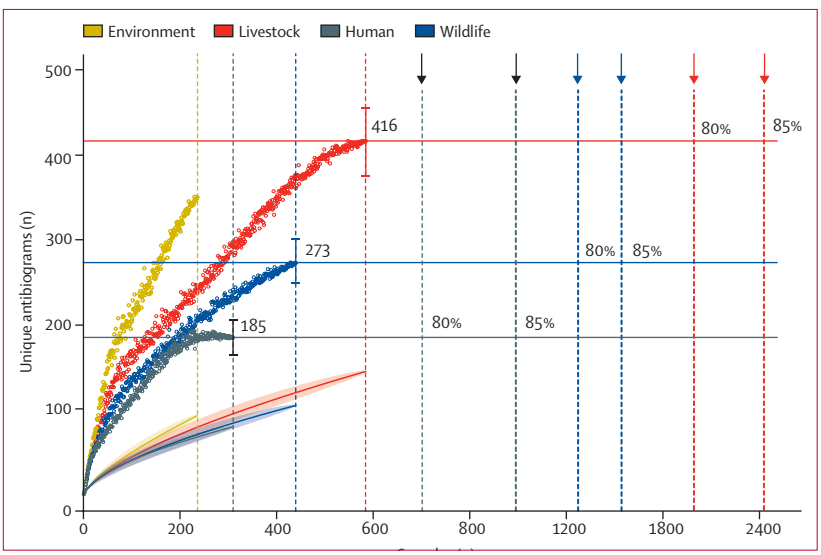

Figure 2: **Asymptotic antibiogram richness estimates for each epidemiological compartment**
Dotted curves indicate Chao2 estimators at every sample point (95% CIs indicated by bars at asymptote). Horizontal lines indicate asymptotic estimate of antibiogram richness for each compartment. Shaded curves indicate species accumulation curves (line represents model fitted values, shaded areas represent 95% CIs). Vertical dotted lines indicate number of samples collected from each compartment. Vertical dashed lines indicate sampling effort required to detect 80% and 85% of the asymptotic estimate for antibiogram richness in each compartment.

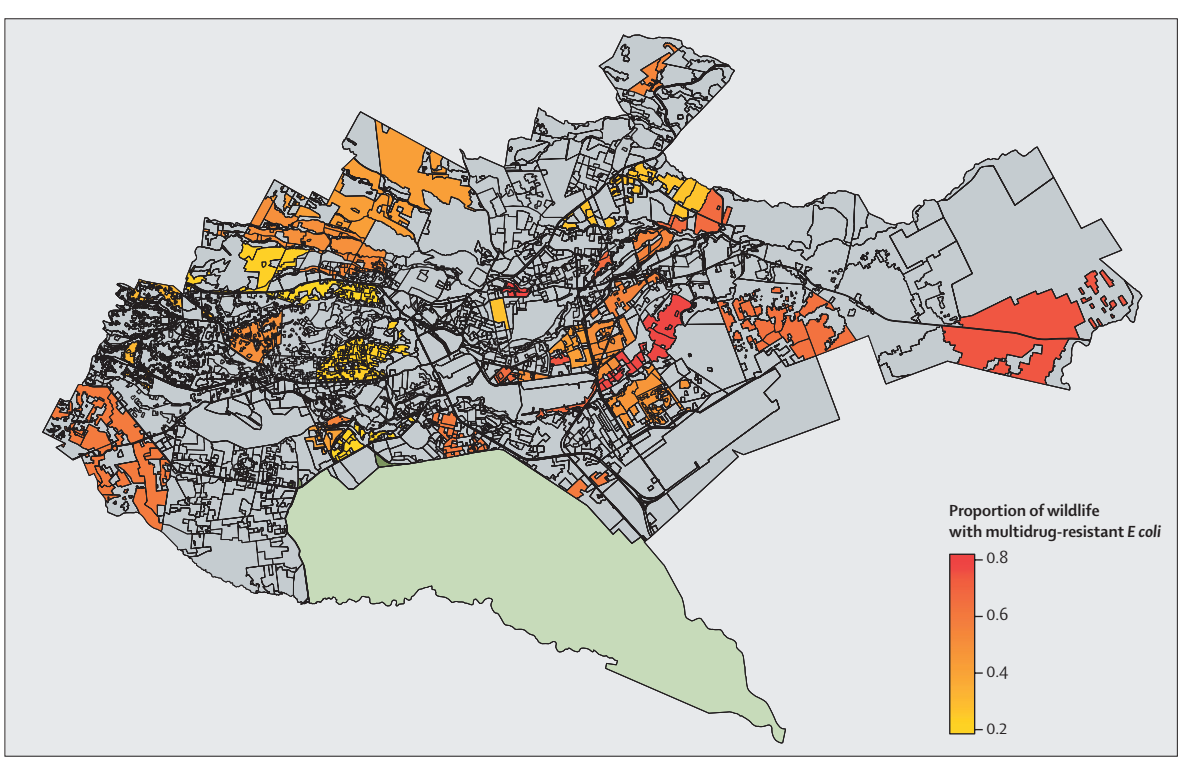

Figure 3: **Proportion of wildlife carrying multidrug-resistant *Escherichia coli*, stratified by the sublocation in Nairobi in which they were sampled**

protocols were standardised between laboratories; specifically, a postdoctoral researcher was responsible for ensuring that these standards were maintained throughout the project. As such, although this variation could have arisen through operator bias, it is likely to have had a limited effect, if any, on our results.

Seed-eating birds and rodents, which are ubiquitous in households across Nairobi and frequently display anthropophilic (human-associated) feeding behaviour, were used as the basis of efforts to understand antimicrobial resistance overlap within households. In any given household, the likelihood of carriage of multidrug resistance in seed-eating birds was best described by increasing numbers of cattle in the household perimeter, and antibiogram length of the human inhabitants (β=3·41, 95% CI 1·42–5·4, p=0·00078; β=1·22, 95% CI 0·16–2·29, p=0·025; R^2 0·3; table 2). The relationship between human antibiogram length and avian carriage of multidrug resistance was affected by whether rubbish was kept within the household perimeter or not (β=4.76, 95% CI 0·76–8·76, p=0·02); keeping rubbish within the perimeter resulted in a stronger relationship between human antibiogram length and avian carriage of multidrug resistance (figure 4A). When manure was kept inside the household perimeter, the probability of carriage of multidrug resistance in seed-eating birds increased with longer antibiogram lengths in livestock, whereas the opposite was true when manure was disposed of externally (figure 4B). The likelihood of multidrug resistance carriage in rodents increased with increasing antibiogram length of human and livestock inhabitants in the household (β=1·31, 95% CI 0·25–2·37, p=0·015; β=0·41, 95% CI 0·03–0·79, p=0·035; R^2 0·42; figure 4C; table 2). Although not statistically significant within the model, keeping both rubbish and manure outside the household perimeter reduced the likelihood of rodents carrying multidrug resistance as human antibiogram length increased.

	Estimate	SE	Z score	p value
Model: MDR carriage in seed-eating birds				
Intercept	−5·4935	2·3398	−2·348	0·019
Total cattle	3·4136	1·0158	3·361	0·00078
Human ABG	1·2222	0·5443	2·245	0·025
Livestock ABG	0·1056	0·2893	0·365	0·72
Manure (outside house)	2·5294	1·4222	1·779	0·075
Garbage (outside house)	4·7585	2·0421	2·320	0·02
Garbage (outside house), human ABG	−1·0513	0·5332	−1·972	0·049
Manure (outside house), livestock ABG	−0·9655	0·4708	−2·051	0·04
Model: MDR carriage in rodents				
Intercept	−4·3039	1·7504	−2·459	0·014
Human ABG	1·3059	0·5383	2·426	0·015
Livestock ABG	0·4085	0·1942	2·104	0·035
Manure (outside house)	2·9078	1·2650	2·299	0·022
Garbage (outside house)	1·4198	1·6627	0·854	0·39
Laboratory (University of Nairobi)	−2·0261	1·1738	−1·726	0·084
Garbage (outside house), human ABG	−1·0043	0·5821	−1·725	0·085
Manure (outside house), human ABG	−0·5909	0·3290	−1·796	0·073

SE=standard error. MDR=multi-drug resistant *Escherichia coli*. ABG=antibiogram length.

Table 2: **Estimated regression parameters, SEs, Z scores, and p values for generalised linear mixed models**

Discussion

We show that urban wildlife species are important components of the environmental pool of resistance to clinically relevant antimicrobials, and through exposure mediated by resource provisioning, could be involved in disseminating clinically relevant resistance across landscapes (appendix). Unlike most previous studies on antimicrobial resistance in wildlife, in which wild animals have been opportunistically sampled,[11] we used an epidemiological study design to compile a large bacterial dataset for investigation burdens of antimicrobial resistance in sympatric wildlife, humans, and livestock, and their shared environment.

High numbers of *E coli* resistant to clinically relevant antibiotics were detected in urban wildlife, including resistance to the more newly developed drugs such as third-generation cephalosporins, and synthetic fluoroquinolones, which WHO considers crucial for human medicine.[25] *E coli* that produce extended-spectrum β-lactamase enzymes, which generally confer resistance to cephalosporins, are a major concern to human and veterinary medicine worldwide, and have been frequently reported in wildlife.[26] However, livestock and environmental compartments (both of which had higher ecological diversity of antimicrobial resistance, higher prevalence of multidrug resistance, longer antibiogram length, and with which humans have more direct contact) yield more potential as routes of human exposure to novel antimicrobial resistance genes in Nairobi. As such, our results are consistent with the hypothesis that wildlife are not a net source of antimicrobial resistance diversity in Nairobi, and probably pose little direct threat to human health in the urban areas. The estimate of total antimicrobial resistance richness in humans was considerably lower than that of all other compartments. This difference, which was robustly supported by statistical estimators, might indicate that, compared with humans, wildlife and livestock are exposed to greater antimicrobial resistance diversity through their closer interaction with the environment.

The vertebrate gastrointestinal microbiome plays a key role in the population structure for genes conferring resistance to antimicrobials, and microbiome composition is directed by an array of factors linked to host genotype,

age, and diet.[27] Although the direct effects of diet and physiological factors on selection for faecal antimicrobial resistance genes could not be assessed, our results are broadly supportive of previous studies that report that anthropophilic omnivores and carnivores have a higher risk of carrying, and potentially spreading, antimicrobial-resistant bacteria.[8] In this study, scavenging birds and water birds had longer antibiograms than all other wildlife species. Antimicrobial resistance-carriage in high proportions of water birds is a common finding in other parts of the world,[28] where, in the absence of natural habitats such as wetlands, these species forage on sewage treatment plants, rubbish dumps, and abattoir viscera ponds. Artificial habitats such as these are considered important routes for the dispersal of human-excreted and livestock-excreted antimicrobial resistance into the environment.[11,29]

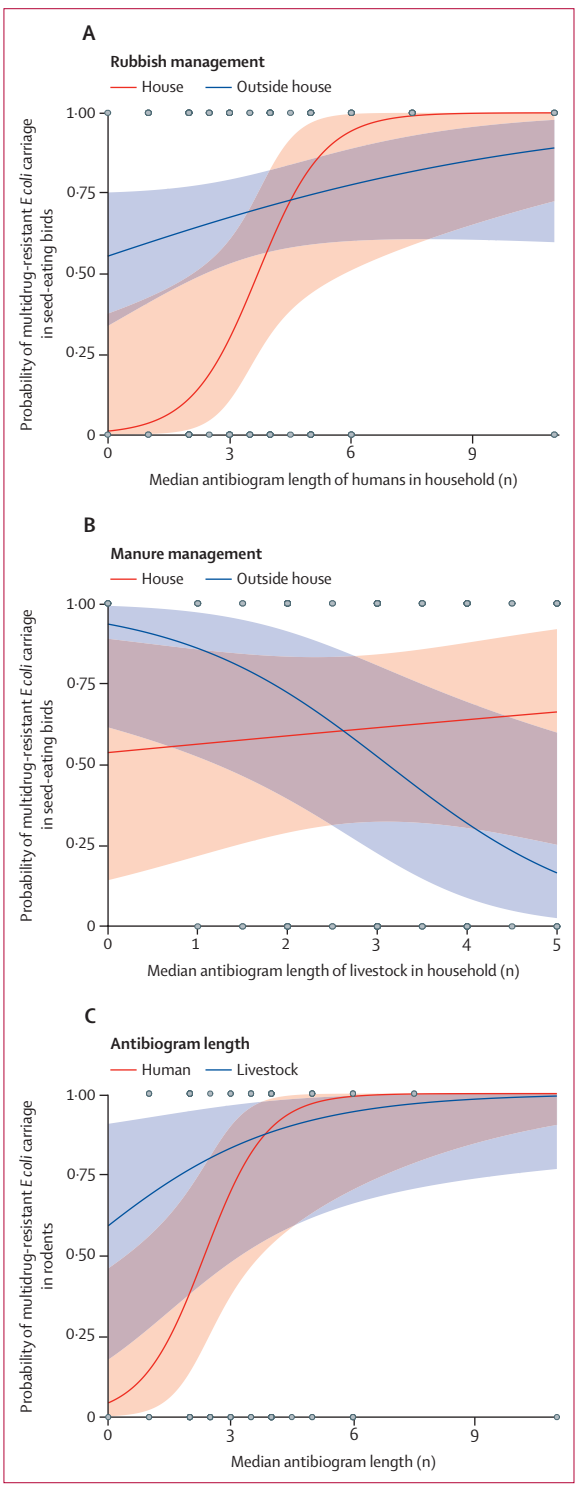

Figure 4: **Fit of the binomial generalised linear mixed effects models relating multidrug-resistant *Escherichia coli* and carriage in birds and rodents to household-level anthropogenic and ecological covariates**
(A) The effects of different rubbish management on the relationship between the probability of multidrug-resistant *E coli* carriage in seed-eating birds and antibiogram length in humans. (B) The effects of different manure management on the relationship between the probability of multidrug-resistant *E coli* carriage in seed-eating birds and antibiogram length in livestock. (C) Human and livestock antibiogram lengths in a household and the probability of multidrug-resistant *E coli* carriage in rodents. All other covariates in the models are kept constant. Shading indicates 95% CIs, and grey points are individual data points.

Within households, increasing likelihood of multidrug-resistant *E coli* carriage in synanthropic wildlife as phenotypic antimicrobial resistance diversity in sympatric livestock and humans also increases suggests transfer of clinically relevant antimicrobial resistance between humans and livestock, and certain wildlife species. These associations were more pronounced for seed-eating birds in the presence of manure and rubbish, indicating that human and livestock waste are conduits for the transfer of antimicrobial resistance between humans, livestock, and peridomestic birds, with the potential for dissemination of antimicrobial resistance phenotypes into the wider environment. Manure can be a reservoir for the amplification of antimicrobial resistance determinants, particularly plasmids.[30] These results support those of other studies[31–33] that have identified the importance of provision of urban resources in bringing wildlife into closer association with humans and livestock, offering new opportunities for disease transmission. However, although our results are suggestive of antimicrobial resistance exchange, transmission cannot be inferred from overlap of phenotypic antimicrobial resistance and, as such, genetic data are required to corroborate the existence of interfaces for antimicrobial resistance exchange, and determine the direction in which bacteria or resistance elements are being transferred. We aim to address this in forthcoming studies.[34] More broadly, wildlife–livestock–human interfaces such as these represent a crucial point for cross-species transmission, and emergence of pathogens into new host populations.[12] Removal of manure and rubbish (sources of anthropogenic resource provision) from households reduced the magnitude of antimicrobial resistance exposure in seed-eating birds, either through limiting wildlife–livestock or wildlife–human contact or reduced exposure of wildlife to sources of antimicrobial resistance.

Complex urban systems such as those of Nairobi are a feature of many lower-middle-income countries, and our findings are therefore broadly applicable to the urban epidemiology of antimicrobial resistance in these countries. High proportions of antimicrobial resistance and multidrug-resistant *E coli* carriage in wildlife could be indicative of environmental antibiotic contamination, and high background levels of antimicrobial resistance in Nairobi's urban environment (supported by our findings

of high phenotypic diversity in environmental samples). Clinically relevant resistance genes were thought to be rare in soils in the preantibiotic era and, as such, it is to be expected that the urban environmental resistome (the collection of resistance determinants present in pathogenic and non-pathogenic bacteria in the soil) in rapidly developing cities such as Nairobi is heavily influenced by human activity.[35] However, interactions between naturally occurring and anthropogenic-derived antimicrobial resistance determinants in bacteria occurring in the broader urban environment, outside urban reanimation units, are poorly understood. The geospatial, temporal, chemical, and biological complexities of urban systems make this a particularly challenging topic of study.

If wildlife exposure to antimicrobial resistance is largely determined by habitat use, targeted surveillance of wildlife that frequent high-risk urban environmental interfaces (where the accumulation of antibiotic residues or other coselecting agents, such as heavy metals, might force the accelerated evolution and fixing of resistance determinants) could be an efficient way to detect clinically important determinants of resistance. To explore the practicality of surveillance in wildlife, the sampling effort required to detect different fractions of the total estimated antimicrobial resistance richness was calculated (figure 2; appendix). To detect all 273 predicted antibiograms in the wildlife species sampled would require an extra 8848 samples, an impractical and expensive task. However, detecting 85% of the total diversity would require a disproportionately lower sampling effort of 1572 samples. Assuming that the diversity of antibiograms in selected wildlife is lower than the total diversity represented by all taxonomic classes of wildlife included in this study, the required sampling effort to achieve an acceptable likelihood of detecting new antibiograms in these species would be much lower. Extending this approach to livestock and humans reveals similar outcomes for surveillance of antibiograms in these compartments (figure 2), suggesting that practical and economically viable surveillance for antimicrobial resistance of public health concern in urban wildlife, livestock, and humans could be achieved through targeted longitudinal surveillance, designed to capture a high proportion of diversity at regular intervals.

Urban ecosystems with high levels of background environmental antimicrobial resistance could act as pools of antimicrobial resistance dissemination to peripheral ecosystems, where the flow of water, and movement of humans, livestock, and wildlife act as vectors for dispersal.[29] Although little is known about how resistance genes are carried and shed by wildlife species,[11] previous studies reporting extended-spectrum β-lactamase *E coli* carriage in migratory wild birds, and carriage of bacteria with resistance to more antibiotics than non-migratory wild birds,[36] indicate that wildlife could have an important role in disseminating clinically relevant antimicrobial resistance across landscapes. Our finding of higher levels of antimicrobial resistance carriage in birds (particularly scavenging birds with large home ranges) than other species suggest that these species could disseminate antimicrobial resistance determinants to neighbouring ecosystems—Nairobi is surrounded by a complex patchwork of high-density human populations, natural areas, forest, and rangelands. Mapping the distribution of multidrug-resistant *E coli* in wildlife by sublocation shows high levels of multidrug resistance carriage extending to peripheral areas of Nairobi, which border rich Savannah ecosystems to the south and east of the city (figure 3). Nairobi National Park, which borders the city to the south, is home to a high density of migratory wildlife species that could disperse antimicrobial resistance genes to more distant areas.[37] Our models for antimicrobial resistance carriage showed a clear east to west gradient, indicating that wildlife antimicrobial resistance diversity is higher in the east of the city, which corresponds to the extreme environmental, ecological, and social gradients that split Nairobi in east to west. Such extreme differentiation within a single city shows the highly complex ecosystem within which the urban epidemiology of antimicrobial resistance is set.

This study has several limitations. Although the prevalence of resistance to individual antibiotics and multidrug-resistant *E coli* carriage in wildlife was high, without comparable datasets from other urban or rural settings it is difficult to say how unique these results are to Nairobi. Studies done in a variety of urban settings, and considering high-risk sites of environmental antimicrobial resistance contamination beyond the household scale, would permit examination of context-specific differences in wildlife antimicrobial resistance carriage and exposure. Because of the effort required to sample wildlife of different species our sample size was small for cryptic taxonomic and functional groups (eg, bats, scavengers, and frugivores or nectarivores). In addition, by only culturing a single isolate from each host, the within-host diversity of antimicrobial-resistant *E coli* was not considered. We made this decision as a necessary, cost-based trade-off between microbiological resolution and sample size. However, the effects of restricted sample size would only act to increase type II error in our results (ie, conservative statistical inference, or missed signal in the data), and are thus unlikely to affect the validity of our findings. More broadly, our focus on mammalian and avian urban wildlife neglects the role of reptiles, aquatic organisms, and invertebrates. Studies investigating the effects of antimicrobial resistance on invertebrates, and their role in carriage and dispersal of resistance elements are warranted given the indispensable role invertebrates play as pollinators, biocontrol agents, and in the degradation and recycling of organic matter in soils.[38,39]

To conclude, carriage of clinically relevant anti-microbial-resistant phenotypes in urban wildlife collected from households in Nairobi is predicted by feeding

ecology, and interaction with humans, livestock, and both human and livestock waste. Even if clinical use is the main driver for the emergence of antimicrobial resistance in humans, environmental compartments such as wildlife can accumulate clinical residues, be reservoirs for novel antimicrobial resistance genes, and have the potential to disseminate resistance determinants across urban landscapes. This potential means that there is a pressing need to consider the ecosystem-wide epidemiology of antimicrobial resistance in urban environments. As Robinson and colleagues[40] speculate, poorly enforced environmental legislation and unregulated antibiotic use might render these factors more pronounced in developing countries. Further studies and targeted surveillance, which take a similarly broad approach to epidemiological compartments, will be required to consider how the genetic determinants of resistance are passed between compartments and disseminated into the wider environment.

Contributors

EMF, TPR, SK, EKK, MEJW, JMH, and MJW conceived of the study. JMH collected field data, analysed data, and drafted the manuscript with MJW, who also contributed intellectually to the manuscript. DM did microbiological testing and contributed intellectually to the manuscript. JMB was involved in the design of data collection protocols, and collected field data. AO and TI collected field data. JK developed laboratory protocols, and did microbiological testing. EMO collected field data, and did microbiological testing. EMF, MEJW, MB, and NJW contributed intellectually to the manuscript. All authors provided comments on the manuscript and gave final approval for publication.

Declaration of interests

We declare no competing interests.

Data sharing

For **data** see http://dx.doi.org/10.17638/datacat.liverpool.ac.uk/729

Data (antimicrobial resistance sensitivity testing datasets, and accompanying metadata) are available via an open access repository held by the University of Liverpool.

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For the **CGIAR Fund Donors** see https://www.cgiar.org/funders/

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ARTICLE

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Deterministic processes structure bacterial genetic communities across an urban landscape

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Land-use change is predicted to act as a driver of zoonotic disease emergence through human exposure to novel microbial diversity, but evidence for the effects of environmental change on microbial communities in vertebrates is lacking. We sample wild birds at 99 wildlife-livestock-human interfaces across Nairobi, Kenya, and use whole genome sequencing to characterise bacterial genes known to be carried on mobile genetic elements (MGEs) within avian-borne *Escherichia coli* ($n = 241$). By modelling the diversity of bacterial genes encoding virulence and antimicrobial resistance (AMR) against ecological and anthropogenic forms of urban environmental change, we demonstrate that communities of avian-borne bacterial genes are shaped by the assemblage of co-existing avian, livestock and human communities, and the habitat within which they exist. In showing that non-random processes structure bacterial genetic communities in urban wildlife, these findings suggest that it should be possible to forecast the effects of urban land-use change on microbial diversity.

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Deterministic (i.e., non-random) processes play a central role in shaping how species communities interact with one-another and their environment[1]. As one such process, urbanisation is characterised by extreme habitat fragmentation, which can have profound impacts on the distribution of host populations and epidemiology of infectious disease. In developing cities such as Nairobi, where urban livestock-keeping is commonly practiced as a result of growing demand for animal-sourced food products[2], wildlife frequently co-exist with humans and livestock, forming interfaces across which infectious diseases can pass[3,4]. Changes in the composition and distribution of these host assemblages likely have important implications for microbial epidemiology, determining how pathogens are distributed within their reservoir, and dictating opportunities for spillover into non-reservoir hosts (such as humans)[5–7]. However, there is little empirical evidence that directly links changes in the function of abiotic and biotic systems to the structure of host communities, and dynamics of microbes living within them. Detecting the processes underlying the structure of microbial communities in wildlife and domestic animal populations would bring us a step closer to developing a predictive framework for pathogen emergence at urban wildlife-livestock-human interfaces[8].

Recent advances in sequencing technology, such as whole-genome sequencing (WGS), offer the potential to study the community of genes carried on mobile genetic elements (MGEs) within prokaryote genomes. MGE-borne genes can be horizontally transferred between organisms via recombination mechanisms, and may confer adaptive functional traits such as antimicrobial resistance (AMR) and virulence[9]. The distribution of MGE-borne genes amongst bacteria can therefore provide insight into the community structure of these micro-organisms, an approach that has been successfully used in conjunction with typing tools and time-scaled evolutionary analyses to infer bacterial transmission between hosts[10–12]. The wealth of genetic data generated by WGS could therefore provide an optimal approach to identify key drivers (such as land-use change) that influence the structure of bacterial populations at high risk wildlife-livestock-human interfaces, and assist in untangling the complexity of epidemiological processes, regardless of the taxonomic distance between hosts.

In this study, we apply principals from evolutionary ecology and molecular epidemiology to investigate whether urban ecological processes (e.g., changes in habitat structure and wildlife communities) and anthropogenic processes (e.g., characteristics of human populations, such as density and livestock keeping) occurring across the city of Nairobi, Kenya, are associated with non-random structuring of wildlife-borne bacterial genetic communities. We consider the diversity of MGE-borne genes as a proxy for the diversity of microbial communities within hosts, with the view that the ability of such genes to move relatively freely between bacterial cells through horizontal gene transfer mimics, to an extent, the movement of directly transmitted pathogens between hosts. For commensal bacteria, determinism would be expected in two classes of MGE-borne genes: those encoding AMR and virulence traits, each of which would be expected to respond differently to urban environmental change. Contamination of the external environment with AMR bacteria excreted from humans and livestock treated with antimicrobials (e.g., through sewage effluent or faeces), is considered an important route of wildlife exposure to AMR[13]. As such, if wildlife-borne bacteria are under higher selective pressure to adopt genes encoding AMR in urban areas where greater volumes of antibiotics are consumed, and antibiotic use is more widespread[14,15], the community structure of MGEs encoding AMR would be hypothesised to respond to changes in human activity and the presence of livestock, rather than natural processes occurring in wildlife communities. In contrast, the diversity of genes encoding virulence traits (for which wildlife-borne bacteria are assumed not to be subjected to such strong anthropogenic selection pressure) would be hypothesised to reflect changes in wildlife host community structure – following the broadly accepted principal that host and microbial community diversity are correlated[5,16], as wildlife host species diversity increases, the pool of virulence-associated MGEs to which they are exposed to should become more diverse.

Adopting the null hypothesis that communities of wildlife-borne bacterial genes are structured by random processes, we test the above expectations by considering variation in the diversity of MGE-borne virulence and AMR genes in commensal *Escherichia coli*, collected from wild birds in household compounds across Nairobi. As likely points of contact (and thus microbial transmission) between vertebrate wildlife, livestock, and humans, household interfaces are chosen as sampling units representative of complex multi-host communities that are widely distributed across a gradient of urban environmental change, and thus suitable for testing our hypotheses. Wild birds are chosen as wildlife hosts in this urban study system, since diverse avian communities distribute widely across urban landscapes[17], demonstrating epidemiological and ecological responses to land-use change[17,18], and interacting closely with livestock and humans[19]. Aside from investigating processes underlying determinism in bacterial genetic diversity, studying the diversity of two sets of genes which may confer adaptive traits to bacteria will enable us to assess whether an association exists between urban land use and the genetic determinants of bacterial selection, with potential implications for human and animal health[9].

Results

Bacterial population structure in avian hosts. Faecal samples ($n = 547$) were collected from 57 avian species in 99 households across Nairobi, that were participating in the UrbanZoo project[20]. Households were selected in such a way that they captured variation in urban land use, wildlife assemblages, human demographics, and livestock-keeping practices across the city (Supplementary Figure 1). A total of 274 *E. coli* isolates, each of which originated from a different individual avian host, were sequenced. Once sequenced, twenty three isolates were removed for being non-*E. coli*, and ten potentially mixed isolates were removed for having a genome size larger than 6 megabases. As such, a total of 241 *E. coli* WGS were considered in further analyses. Genes carried on MGEs, which were known to encode virulence ($n = 63$) or AMR ($n = 47$), were identified in 98% ($n = 236$) and 44% ($n = 107$) of these *E. coli* respectively. *E. coli* population structure across hosts was explored using multi-locus sequence typing (MLST). 128 unique sequence types (STs) were identified, representing a high genetic diversity of *E. coli* in avian samples across the city (Supplementary Figure 2). No sequence type was assigned to 18 isolates that carried at least one novel allele not included in the (MLST) database. The most common STs (ST10, ST155 and ST48; those appearing in > 5% of isolates) were randomly distributed across host functional groups, and not associated with the diversity of MGE-borne AMR and virulence genes in each isolate (Fisher's Exact test: $p = 0.18$; Kruskal–Wallis test AMR genes: $X^2 = 7.17$, $P = 0.62$, $df = 9$; Kruskal–Wallis test virulence genes: $X^2 = 10.4$, $P = 0.11$, $df = 6$).

To test whether microbial genetic communities in avian hosts were deterministically structured in association with the environmental conditions and structure of host communities at household interfaces within which avian hosts resided, the α-diversity of each set of genes (counts, thus representing richness of virulence or AMR genes) was calculated for individual hosts, and

regressed against ecological and anthropogenic characteristics of households using generalised linear mixed effects models (GLMMs). Ecological and anthropogenic factors that were selected as indicators of variation in household environmental conditions, and used as fixed effects in the models, included: α-diversity (richness) of avian species present, biotic habitat diversity, artificial land-use cover (%), wealth indices, livestock-keeping status of each household, livestock density, and human density. Variation in bacterial genetic diversity introduced by differences in the feeding ecology and ranging behaviour of avian hosts was accounted for by including membership of avian hosts to epidemiologically relevant functional groups, and allometrically scaled estimates of each species home range, as fixed effects in each model. Two isolates for which host identity could not be confirmed were excluded from the statistical analyses (bringing the total number of genomes on which analyses were performed to $n = 239$).

Virulence gene diversity, avian host communities and habitat. We found that the diversity of virulence genes present in birds varied between host functional groups, and increased with α-diversity of household avian communities (marginal R^2: 0.08, Table 1). However, the relationship between virulence gene and avian diversity varied between functional groups, with a significant positive relationship only being present in invertebrate-eating birds (Fig. 1). Habitat diversity and livestock density showed significant inverse relationships with virulence gene diversity (GLMM: $\beta = -0.65$, 95% CI $= -1.17$–-0.13, $P < 0.05$; GLMM: $\beta = -0.69$, 95% CI $= -1.34$–-0.07, $P < 0.05$). To further explore determinants of virulence gene diversity in seed-eating birds (which, as synanthropic species, constituted the largest and most well-distributed avian functional group), a separate Poisson-distributed GLMM was built considering only the genetic diversity of sequences derived from this functional group ($n = 152$). This also had the effect of removing variation associated with functional group membership. Once other functional groups had been excluded, habitat diversity had a significant inverse relationship with diversity of virulence genes in seed-eating birds; as habitat diversity decreased, diversity of virulence genes in seed-eating birds increased (GLMM: $\beta = -0.76$, 95% CI $= -1.3$–-0.23, $P < 0.01$; marginal R^2: 0.06).

AMR gene diversity and assemblages of livestock and humans. Determinants for the diversity of genes encoding AMR were investigated in a similar way, utilising the same set of avian *E. coli* isolates and household explanatory variables used for virulence genes. The best-fitting model was a zero-inflated hurdle model (with a truncated Poisson error distribution), in which the presence or absence of AMR genes (the zero-inflated component) and increasing diversity of AMR genes (the conditional component) were modelled separately. The conditional model demonstrated that α-diversity of AMR genes was significantly associated with increasing human density, but only in households keeping livestock (GLMM: $\beta = 0.99$, 95% CI $= 0.34$–1.65, $P < 0.01$; Table 1). This was supported by the zero-inflated component, which showed a significant negative association between the probability of AMR genes not being detected in avian-borne *E. coli* and increasing human density (GLMM: $\beta = -2.11$, 95% CI $= -3.83$–-0.45, $P < 0.05$; Table 1). To test whether the interaction between human density and livestock keeping was dependent upon avian host functional-group membership, the same model was fitted independently for isolates derived from seed-eating ($n = 152$) and non-seed-eating birds. This indicated that the relationship between AMR gene diversity, livestock keeping and human density was only present for seed-eating birds (GLMM: $\beta = 0.91$, 95% CI $= 0.17$–1.65, $P < 0.05$; Fig. 2), and that the likelihood of detecting AMR genes increased with the presence of livestock, and increasing human density (Table 1). To explore these relationships further, the fixed covariate livestock keeping was replaced with livestock density (correlation prevented both from being fitted in the same model). The resulting model showed a positive, although non-significant, association between livestock density and diversity of AMR genes in seed-eating birds (GLMM: $\beta = 0.53$, 95% CI $= -0.07$–1.13, $P = 0.08$; Table 1).

Gradients of microbial genetic diversity across Nairobi. Microbial genetic diversity was framed against city-wide variation in host community structure at household interfaces, by relating the outcomes of our models to the results of an unconstrained principal components analysis (PCA) that was used to decompose variance attributed to avian diversity, livestock density, and human density within households across Nairobi. The first principal component (PC1) accounted for 72.9% of variation, clearly separating households with high avian diversity from households with high human and livestock density. Relating city-wide trends in host community structure to associations between diversity of virulence genes and avian diversity, and diversity of AMR genes and livestock and human density, reveals opposing epidemiological gradients of bacterial genetic diversity across Nairobi (Fig. 3a).

Discussion

Understanding the influence of environmental change on the diversity and distribution of microbial communities in wildlife is of fundamental importance to understanding how zoonotic diseases spillover into humans. Here, spatially explicit data on land use, the ecology of host populations, and high resolution microbial sequencing in individual hosts, is linked to explore this question across a developing city. We found that deterministic forces, both ecological (wildlife species assemblages and biotic habitat diversity) and anthropogenic (human and livestock density), operating across the urban landscape of Nairobi are associated with variation in the structure of bacterial genetic communities within avian host communities.

For virulence genes, the species richness of host communities was positively correlated with the diversity of genes present in *E. coli* isolates, with increases in avian diversity being associated with a higher diversity of virulence genes within their *E. coli*. This follows an expected pattern for communities of hosts and their microbial diversity. Assuming each vertebrate host harbours at least some *E. coli* bearing unique virulence genes, increasing vertebrate species diversity will increase the diversity of virulence genes circulating in the population[21] (reviewed by Ostfeld & Keesing[16]). Our results are consistent with the hypothesis that, in this study system, increased vertebrate diversity results in avian-borne *E. coli* acquiring a greater diversity of virulence genes, because of exposure to a larger pool of available genes in the vertebrate host community. The composition and size of this pool of available genes would be hypothesised to vary across a gradient of urban land use, as the structure of avian communities change in response to the changes in habitat structure and biotic resource provision. However, our results also suggest that the relationship between microbial and host community diversity is subject to variation in host functional ecology. For frugivorous birds, which had higher mean diversities of virulence genes, virulence gene diversity was negatively correlated with avian diversity, perhaps because their exposure to *E. coli* harbouring novel virulence genes is driven by dietary exposure rather than transmission between hosts.

Table 1 Estimated regression parameters, standard errors, z-values and *P* values for optimal generalised linear models used in this study, modelling the diversity of avian-borne *E. coli* virulence and antimicrobial resistance (AMR) genes against household environmental variables

Model Terms	Estimate	Std. Error	z value	*P* value
Model 1: Virulence genes, All avian functional groups				
Intercept	1.0542	0.4432	2.379	<0.05
Avian Species Richness	0.0447	0.0224	2	<0.05
Fruit/Nectar	2.321	0.831	2.793	<0.01
PlantSeed	0.5413	0.4009	1.35	0.18
Omnivore	0.7884	0.5745	1.373	0.17
Livestock Density	−0.6939	0.3202	−2.167	<0.05
Habitat Diversity	−0.6465	0.2598	−2.488	<0.05
Avian Species Richness:Fruit/Nectar	−0.1202	0.0433	−2.778	<0.01
Avian Species Richness:Seedeater	−0.0417	0.0236	−1.768	0.08
Avian Species Richness:Omnivore	−0.0478	0.0325	−1.468	0.14
Model 2: Virulence genes, Seed-eating birds only				
Intercept	1.8383	0.2152	8.54	<0.001
Habitat Diversity	−0.7587	0.2698	−2.812	<0.01
Livestock Density	−0.6564	0.3365	−1.95	0.05
Model 3: AMR genes, All avian functional groups (Zero-inflated hurdle, truncated Poisson)				
Conditional model				
Intercept	1.9068	0.1562	12.204	<0.001
Livestock kept within household	−0.3171	0.1704	−1.86	0.063
Human Density	−0.41413	0.2601	−1.593	0.111
Livestock-keeping:Human Density	0.9948	0.3332	2.986	<0.01
Zero-inflation model				
Intercept	1.3175	0.4637	2.841	<0.01
Livestock kept within household	−0.8217	0.5202	−1.58	0.114
Human Density	−2.1407	0.8627	−2.481	<0.05
Livestock-keeping:Human Density	0.3796	1.3059	0.291	0.771
Model 4: AMR genes, Seed-eating birds only (1) (Zero-inflated hurdle, truncated Poisson)				
Conditional model				
Intercept	1.8531	0.2073	8.938	<0.001
Livestock kept within household	−0.2788	0.2227	−1.252	0.211
Human Density	−0.3355	0.304	−1.104	0.2698
Livestock-keeping:Human Density	0.9107	0.3768	2.417	<0.05
Zero-inflation model				
Intercept	1.3989	0.6002	2.323	<0.05
Livestock kept within household	−1.4189	0.6706	−2.116	<0.05
Human Density	−2.2329	0.9883	−2.259	<0.05
Livestock-keeping:Human Density	1.8476	1.4079	1.312	0.1894
Model 5: AMR genes, Seed-eating birds only (2) (Zero-inflated hurdle, negative Binomial)				
Conditional model				
Intercept	1.58251	0.0923	17.195	<0.001
Livestock Density	0.53141	0.30462	1.745	0.081
Zero-inflation model				
Intercept	0.2055	0.2295	0.895	0.371
Livestock Density	−1.1402	0.9043	−1.261	0.207

For two-stage hurdle models (Models 3–5), a positive contrast in the conditional model represents a higher abundance, whilst a positive contrast in the zero-inflated model indicates a higher chance of absence

The results of this study also indicate that differences in the response of wildlife species to changes in urban land use could play a part in determining how microbial genetic diversity is related to host community diversity. For example, the diversity of virulence genes in *E. coli* derived from seed-eating birds, which show more synanthropic behaviour than other functional groups, was predicted by changes in biotic habitat diversity rather than avian community diversity: in seed-eating birds, increasing virulence gene diversity was linked to decreasing biotic complexity of habitats. Further evidence for the role of host taxa in shaping the response of microbial genetics to variation in urban land use was provided by considering MGEs conferring AMR in *E. coli*. Increasing human and livestock density were associated with higher AMR gene diversity in avian-borne *E. coli*, but this only applied to isolates recovered from seed-eating birds. Importantly, the significant relationship between AMR gene diversity and human density was only found amongst household which kept livestock, providing evidence to suggest that households may act as an interface for the exchange of genes encoding AMR between livestock and wild birds. Livestock and human density could therefore be responsible for influencing the diversity (or pool) of AMR genes present and/or promoting contact with synanthropic wildlife, resulting in spillover of bacteria and/or their genetic elements from livestock to wildlife within household compounds.

Our findings are important for several reasons. First, they point towards the presence of opposing epidemiological gradients for AMR and virulence genes across the urban landscape, in which communities of mobile microbial genes are correlated with changes in the richness and density of vertebrate host communities (which may be confounded by the ecological traits of the host within which that organism resides) (Fig. 3a). Although the

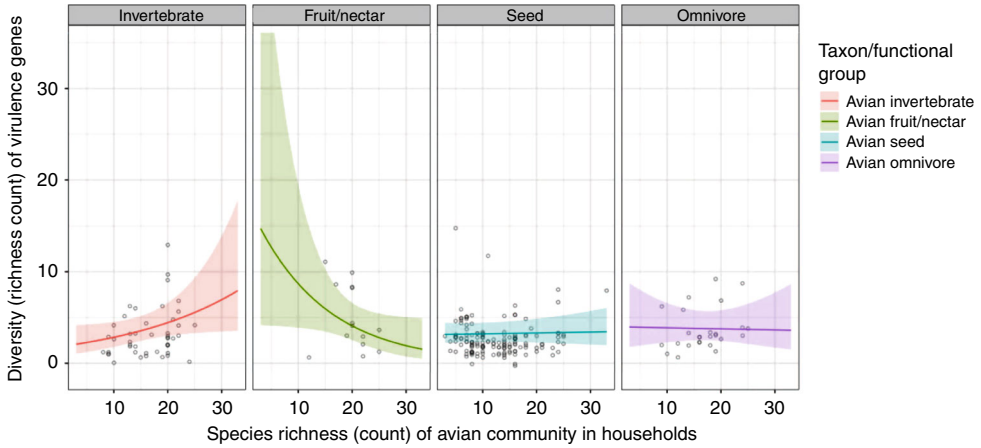

Fig. 1 Virulence gene diversity and avian species richness. Fit of the Poisson GLMM, modelling how diversity (richness) of virulence genes in avian hosts ($n = 239$) varies as a function of avian host community richness and functional group membership. Coloured lines represent different avian functional groups, and shading on either side of each line represents 95% confidence intervals

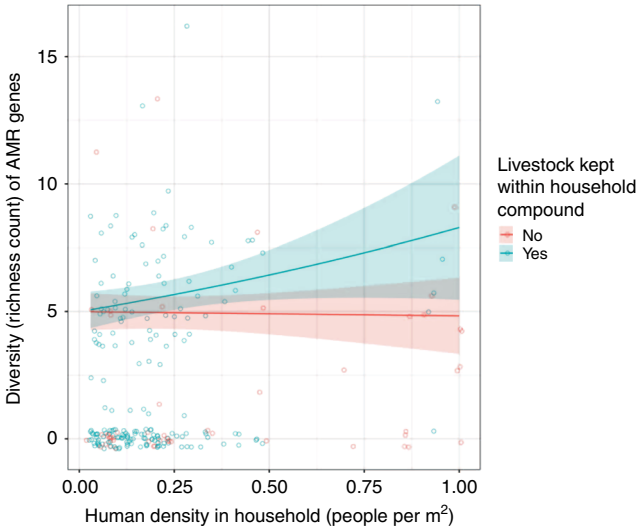

Fig. 2 Antimicrobial resistance (AMR) gene diversity and human density. Fit of the zero-inflated hurdle model, modelling how diversity of AMR genes in seed-eating birds increased with human density, when livestock were part of the vertebrate host community at household interfaces. Coloured lines represent the presence or absence of livestock in households, and shading on either side of each line represents 95% confidence intervals. A subset ($n = 152$) of *E. coli* isolates were included in this analysis

horizontal exchange mechanisms involved in the transfer of these genes are unlikely to directly mimic the dynamics of microbial transmission, such deterministic patterns might also be displayed by microbial communities subject to the same changes in host community structure. For example, abundance of hosts has been linked to parasite species richness in a number of previous studies[22,23], and increasing diversity of helminth parasitism in Southeast Asian murids has been positively correlated with a gradient of anthropogenic habitat change[24].

Second, our results provide evidence for a mechanism by which anthropogenic processes tied to variation in urban land use result in spillover of MGEs (and potentially microbes) between vertebrate host compartments at wildlife-livestock-human interfaces (Fig. 3b). And third, considering variation in avian community assemblage and the form of human and livestock populations as indicators of differing ecological and anthropogenic processes, our findings suggest that processes associated with urbanisation can simultaneously exert very different forms of genetic selection (e.g., exposure to diverse pools of virulence or AMR genes) on the same species of bacteria. This could have important implications for public health. For bacterial organisms such as *E. coli*, exposure to larger pools of genetic diversity that promote uptake and fixing of AMR genes can confer adaptive advantages such as drug resistance[25], whilst acquisition of virulence determinents in the accessory genome has been frequently implicated in the emergence of pathogenic lineages of *E. coli*. Divergence associated with horizontal gene transfer between closely related microbial strains can lead to the emergence of novel pathogens[26,27].

In this study, high resolution genetic data collected as part of a structured epidemiological study, was used to study bacterial epidemiology in a multi-host urban system. Whilst the scale of sampling conducted in this study (representing sympatric wildlife, livestock and human communities along a gradient of urban land use) provided the opportunity to explore hypotheses that, until recently could not have been tested, this dataset is not without epidemiological limitations, and the results presented in this study should be interpreted with the following considerations in mind. To better contextualise the transfer of MGE-borne genes, in particular those borne on plasmids, longer read sequencing (e.g., PacBio) would provide an advantage over short-read Illumina data in making epidemiological inferences[28]. However, the focus of this study was on patterns of diversity in terms of gene presence or absence rather than characterising individual genes and the genetic context of their transfer. The sensitivity of commensal *E. coli* in identifying transmission pathways for other pathogens should also be considered with caution. Differences in characteristics (such as shedding rates and effects on host behaviour) between commensal and pathogenic organisms may have epidemiological consequences that reduce their representation of one another. In addition, by only sequencing a single *E. coli* isolate from each host, the within-host genetic diversity of *E. coli* was not considered. Previous molecular studies on *E. coli* (albeit it in different hosts, and using lower resolution sequencing technology), have demonstrated considerable within-host diversity across vertebrate taxa[29–31]. However, the decision to sequence a single isolate from each host was made as a necessary, cost-based trade-off between genetic resolution, depth of sampling *E. coli* genetic diversity within each individual, and the number of unique wildlife individuals from which samples could be included. Under sampling within-host diversity would only be likely to lead to a signal being missed, rather than changes to the positive results that we report in this study.

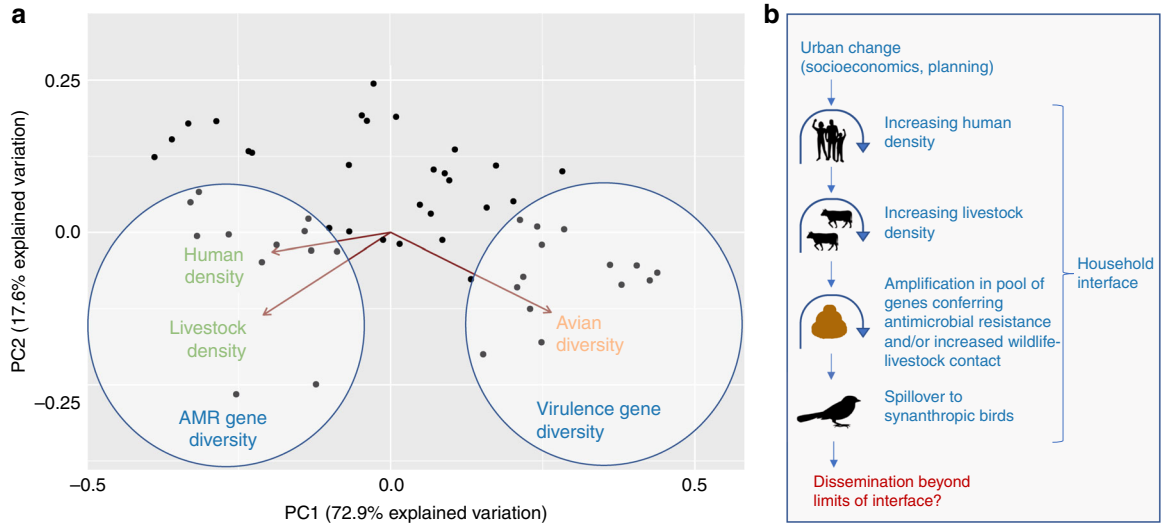

Fig. 3 Epidemiological gradients in MGE diversity, and pathways to AMR spillover under urban change. **a** Diagrammatic representation of how epidemiological gradients in diversity (richness) of virulence and AMR genes in avian-borne *E. coli* overlay on broad-scale trends in host community characteristics at urban household interfaces. The characteristics of host communities are represented in the form of a principal components analysis (PCA), performed on avian diversity (species richness), human density and livestock density in households. PC1 accounts for most variation, separating households with high avian diversity from households with high human and livestock density. Associations between the diversity of virulence and AMR genes, and avian diversity human density and livestock density are indicated by circles overlaid onto the PCA biplot. **b** Schematic illustrating possible processes leading to spillover of AMR genetic determinants between livestock and synanthropic birds at household interfaces

In demonstrating that it is possible to link epidemiological processes in wildlife to environmental drivers across urban landscapes, this study has taken the first step towards forecasting the effects of urban land-use change on disease emergence within a developing city. Whilst the focus of this study was on wildlife, understanding how urban environmental change structures microbial communities in human and livestock hosts is equally important, and extending analysis of the diversity of genes carried on MGEs to humans and livestock would provide valuable insight into the epidemiological responses of these compartments to variation in land use. By considering genetic diversity in a single species of *Enterobacteriaceae* as a proxy for parasite diversity, this study has necessarily taken a reductionist approach to address important hypotheses that otherwise could not have been answered using this dataset. The limitations in using a model organism such as *E. coli* could be addressed through utilising recent advances in metagenomics, which permit sequencing of bacterial and viral microbiomes, to characterise the structural response of microbial communities to the environmental drivers of urban land-use change. Such methods could be utilised in the future to understand how changes in microbial diversity, and the uptake and fixing of genes by pathogens, translate to emergence and manifestation of clinical disease in wildlife, livestock and humans.

Methods

Animal care and use. The collection of data adhered to the legal requirements of the country in which the research was conducted. Wildlife were trapped under approval of an International Livestock Research Institute (ILRI) Institutional Animal Care and Use Protocol (2015.12).

Human ethics statement. Questionnaire data was collected under ILRI Institutional Research Ethics Committee approval (2015-09), and prior informed consent was gained for each individual participating in the project.

Study design. The study focused on household livestock keeping as it represents a point of largely unmanaged, intense contact between synanthropic wildlife, livestock and humans. Faecal samples ($n = 547$) were collected from 57 avian species from 99 households across Nairobi, that were participating in the UrbanZoo project[20]. The UrbanZoo project, based in Nairobi, Kenya from 2012–2017, aimed to utilise a landscape genetics approach to understanding the movement and sharing of pathogens in a major developing city. A key component of this project, within which this study was nested, was the '99 household project', which focused on informal livestock keeping practices in urban households as a route of zoonotic disease emergence in humans. As such, households were selected with the aim of maximising the spatial distribution and diversity of livestock keeping practices across Nairobi, and were chosen to capture three main criteria: socio-economic diversity, population distribution and livestock keeping practices. Geospatial mapping data, generated as part of a technical report produced by Institut Français de Recherche en Afrique (IFRA), was used to identify 17 classes of residential neighbourhood in Nairobi based on physical landscape attributes, which were subsequently verified by 817 household questionnaires[32]. Each of the 17 classes of neighbourhood were then ranked by average income and reduced into seven wealth groups. Administrative sublocations were mapped onto each wealth group, identifying a total of 70 possible sublocations, for which dominant wealth groups were calculated by extracting the proportion of population belonging to each neighbourhood class within the sub-location boundaries (Supplementary Table 1). A total of 33 sublocations were selected to be included in the study, with the number of sublocations belonging to each wealth group chosen proportionately to the population density and the variety of neighbourhood classes in each of the seven wealth groups. Final selection of individual sublocations was aimed at maximising areas with high livestock densities, whilst ensuring coverage of other neighbourhood classes and geographical spread.

For each sublocation, three geographical points were selected at random within the dominant housing type. The order in which sublocations were visited was randomised. Local officials assisted in the recruitment of a household closest to each geographical point, to obtain two livestock keeping and one non-livestock keeping household per sublocation (a total of 99 households, 66 of which kept livestock). Households had to meet strict inclusion criteria of keeping either large ruminants (cattle), large monogastrics (pigs), small ruminants (goats/sheep), small monogastrics (poultry/rabbits), or no livestock species. To ensure an equal sample of both cattle and pig-keeping households, the combination of livestock keeping households represented in each sublocation was randomised, and had to consist of either large ruminant and small monogastric, or large monogastric and small ruminant species. For sublocations in which households keeping large ruminant or large monogastric species were absent, a replacement household keeping either small monogastic or small ruminant species was recruited. Sampling of households took place between September 2015 and September 2016.

Wildlife trapping and ecological surveys. A dedicated field team was responsible for collecting data on humans, livestock and wildlife in each household, consisting of veterinarians, animal health technicians and clinicians. Mist nets were set at dawn to trap birds, with nets being positioned outside the house and around livestock keeping facilities. Once caught, all birds were live-sampled in the field under manual restraint, before being released unharmed. Morphometric data were collected for identification purposes, and a suite of biological samples (including

faeces if available, or a cloacal swab) were collected from each animal. Due to large variation in the size of household compounds, trapping effort (i.e., number of mist nets placed per trapping session) was maintained such that it was proportional to the size of the household compound. Ecological surveys were used, alongside trapping data, to estimate the diversity of avian species present within households. Avian species counts (presence/absence) were conducted by a trained ornithologist from the National Museums of Kenya, in which species were identified based on audio-visual identification over a 20-minute period spent walking transects of each household compound. Surveys were conducted between 6:30am and 9:30am, over the course of two months in the dry season, ensuring that bird activity and weather conditions were constant. The species richness of avian communities (α-diversity: the total number of avian species recorded in a household) was calculated for each household. Avian species were also grouped into five functional groups, deemed relevant for the epidemiology of a directly transmitted gastrointestinal parasite such as *E.* coli; plant/seed-eating, omnivorous, fruit/nectar-eating, invertebrate-eating, vertebrate/fish-eating/scavenger. Allocation of avian species to functional groups was based upon the EltonTraits database[33]. Home range estimates for all avian species were calculated by allometric scaling of body weight[34]. Scaling factors published for functionally different birds by Ottoviani et al.[35] were used, and species mean body weights were either collected during sampling, or sourced from published datasets when unavailable[36].

Household questionnaires. A nominated member of each household completed a questionnaire, detailing *i)* livestock ownership, management, sourcing, sales and antimicrobial use, and *ii)* household composition and socio-economic data. Abundance (counts) of livestock species and humans were derived from this data for each household. Dividing livestock and human abundance by household area (meters^2, as measured using ArcGIS) generated an estimate of density of livestock and humans. Household composition and socio-economic data were used to generate wealth and ruralness indices for each household sampled[20]. These indices were calculated based on methods used to create the Demographic and Health Surveys (DHS) wealth index, which is derived from a Principal Component Analysis (PCA) of easily measurable households assets (such as access to water, construction materials and ownership of livestock)[37]. A modification was made to the original set of household assets included in the DHS index to better capture household variation in Nairobi. All field data was recorded using Open Data Kit (ODK) Collect software (Hartung et al., 2010), on electronic tablets, and uploaded to databases held on servers at the International Livestock Research Institute (ILRI).

Land-use classification. Nairobi is characterised by a large variety of land use. Land use comprises the biotic and abiotic niches within which hosts exist, and was classified for each household. The boundary of each household compound was drawn in ArcMap, and a 30 m buffer created around the perimeter of each compound to represent the landscape surrounding it. A buffer of 30 m was chosen to reflect home range of common urban rodent species (*Mus* and *rattus* spp., estimates of which vary from 1 m to 30 m)[38,39]. Visual classification of land-use types within the compound and buffer area were conducted at 1:500 scale on a 1 m resolution ESRI World Imagery satellite-image available in ArcGIS 10.5 (ESRI). Characterisation of ecological characteristics along a perimeter around the household compound was considered as important, because the ecological setting within which the household exists extends beyond the boundaries of the compound. The extent to which this influential area of habitat outside the compound extends is unknown, and as such it was standardised across study sites. Within the boundary, the areas of nine different land-use types were visually identified and sketched as polygons; water-body, wetland, crops, mature trees, shrubs, grassland, bare ground, artificial ground and rubbish (descriptions for each of these are summarised in Supplementary Table 2). The total area of classified land-use types at each site were calculated and expressed as proportions. Ecological land-use types (all except bare ground, artificial and rubbish) were used to calculate Simpson's diversity index, which considers both habitat richness, and an evenness of abundance among the land-use types present at each site. This index was created to represent the diversity of living (biotic) habitat niches available to wildlife within households, and ranged from 1 (maximum heterogeneity) to 0 (only a single category of biotic land use present). All classification was undertaken by J.M.H. who was familiar with the landscape at each site, and subsequently ground-truthed by revisiting sites.

Microbiological testing. All swabs and fresh faecal samples were placed in Amies transport media and transported on ice to one of two laboratories (Kenya Medical Research Institute (KEMRI) or University of Nairobi (UoN)). Samples were enriched in buffered peptone water for 24 hours, and plated onto eosin methylene blue agar (EMBA). Plates were incubated for 24 hours at 37 °C, after which five colonies were selected from each EMBA plate. After a further sub-culture on EMBA to purify the isolates, the pure isolates were sub-cultured on Müller-Hinton (MH) agar and archived at −80 °C in cryovials containing Soy broth supplemented with 15% glycerol.

Next-generation sequencing. A single colony was picked from each original sample (referred to as an isolate) and biochemical tests (triple sugar iron agar, Simmon's citrate agar, and motility-indole-lysine media) were run for identification as *E. coli*. DNA was extracted from bacterial isolates using commercial kits (Purelink® Genomic DNA Mini Kit, Invitrogen, Life Technologies, Carlsbad, California) and transported under licence to The Wellcome Trust Centre for Human Genetics, Oxford, UK. Whole genome sequencing (WGS) was carried out at the Wellcome Trust Centre for Human Genetics on the Illumina HiSeq 2500 platform. 150 base-pair paired-end reads were generated and short-read WGS data were pre-processed using an automated protocol developed by the Modernising Medical Microbiology Oxford (MMM) Group to: (i) perform standard quality control checks using fastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) with default settings; (ii) trim reads to remove remnant adaptor sequences using bbduk[40] (parameters: minoverlap = 12, k = 19, mink = 12, hdist = 1, ktrim = r) and (iii) perform a Kraken[41] speciation analysis against with an in-house database of bacterial reads downloaded from the NCBI sequence read archive (www.ncbi.nlm.nih.gov/sra/), with an automated step for removal of contaminant (non-bacterial) reads. De novo assembly was performed using SPAdes v3.6[42] (parameters:–careful, -t 1,–phred-offset 33). The assemblies were run through the batch upload mode of the Centre for Genetic Epidemiology web interface hosted by the Technical University of Denmark (https://cge.cbs.dtu.dk/services/cge/) which performs speciation analysis[43], multilocus sequence typing (MLST)[44], detection of resistance genes[45] and detection of virulence genes[46]. The threshold of AMR gene detection was set to 90% identity and 60% coverage, as this is shown to be the optimal threshold for this method. A 60% coverage threshold was used to ensure that AMR genes spread over two contigs, and/or located on the edge of the contig, were not missed[45]. Virulence genes were identified using VirulenceFinder with 90% minimum match and 60% minimum length. Samples deemed as non-*E. coli* on the basis of the speciation analysis with kmerFinder[47] in the Centre for Genetic Epidemiology pipeline were excluded from further analysis. Potentially mixed *E. coli* samples were identified as those with an unusually large assembly size (greater than 6 megabases (Mb)) and were removed from the dataset. Supplementary Table 3 details the QC and assembly metrics of the 241 *E. coli* isolates included in the study.

Statistical analyses. All statistical analyses were conducted using R v3.3.2[48]. The response variables diversity of virulence and AMR genes, were regressed against explanatory variables in generalised linear mixed effects models (GLMMs). Isolates for which AMR or virulence genes were not detected were included in these analyses. To address the fact that genes co-mobilised on the same MGE might not represent independent acquisition events without having access to long-read sequencing (which would enable identification of the location of genes on plasmids), we combined all pairs of genes with 100% co-occurrence (e.g., bfpA and perA). To account for the dependency structure of the data, the household and sublocation in which samples were collected were included as nested random effects. To account for the relationship between bacterial population structure and MGE diversity, we also included a measure of bacterial population structure as a random effect in each model. Due to high MLST diversity in the dataset (128 unique STs, and 18 novel STs), sequence type could not be included as a random effect, and as such, each isolate was assigned to a less stringent cluster using the BURST algorithm, on the basis of 3 rather than 7 genetic loci. This composite measure of genetic structure was included as a random effect in each model. Models of virulence gene diversity were fitted with a Poisson distribution in the R package lme4[49]. Preliminary data exploration indicated substantial zero-inflation in the response variable α-diversity of AMR genes (i.e., many samples where no AMR genes were detected), and as such a zero-inflated Poisson model (ZIP) was initially fitted to the data (56% of data comprising the response variable were zeros). However, residuals from the optimal ZIP model obtained through step-wise selection showed considerable overdispersion (dispersion statistic: 3, a value of 1 is considered to represent adequate statistical dispersion). Dispersion parameters were stabilised by fitting zero-inflated mixture and hurdle models available in the R package glmmTMB[50] to the data. These classes of model are frequently used to model zero-inflated count data in ecological datasets. The fit of these models were compared using Akaike's information criteria (AIC).

Optimal models were constructed using stepwise, backwards elimination from the full model based upon (AIC). Significance of model terms were tested by the maximum likelihood test, and the fit of each model was reported as marginal regression coefficients of multiple determination (marginal R^2) where possible. Model assumptions were verified by plotting residuals versus fitted values, and by assessing models for overdispersion. Non-linear relationships were checked by fitting a generalized additive model (GAM) between the response and explanatory variables, featuring a nonlinear smoother, in R package mgcv[51]. The residuals were also assessed for spatial dependency by plotting them against geographic coordinates, and examining the results of a semivariogram.

An unconstrained principal component analysis (PCA), was performed on avian diversity, livestock density, and human density within households across Nairobi, in the R package vegan[52].

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data (AMR and virulence gene datasets, and accompanying metadata) are available via an open access repository held by the University of Liverpool (http://dx.doi.org/10.17638/datacat.liverpool.ac.uk/738). All sequencing reads are available on the European Nucleotide Archive, under Project ID: PRJEB32607.

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Author contributions

E.M.F., T.P.R., S.K., E.K.K., M.E.J.W., M.J.W. and J.M.H., conceived the study. J.M.H. collected field data, performed all data analysis, and drafted the manuscript. J.M.B. was involved in the design of data collection protocols, and collected field data. A.O. and T.I. collected field data. J.K. developed laboratory protocols, and conducted microbiological testing. M.J.W., D.M. and H.P. designed and performed bioinformatic analysis. M.B. contributed intellectually to the study design and analysis. All authors provided comments on the manuscript and gave final approval for publication.

Additional information

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